GENETICS

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BEARING ON HEREDITY AND VARIATION

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THE INTERRELATION OF PLASMAGENES AND CHROMOGENES IN POLLEN PRODUCTION IN MAIZE

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Received January 24, 1950

AN INHERITED condition of pollen abortion is frequently found in many species of plants. In maize 20 genes for male sterility are listed by EMERSON, BEADLE and FRASER (1935). Male sterile-1 reported by SINGLETON and JONES (1930) is on chromosome 6 closely linked with the *Vy* endosperm locus. All of the gene steriles described are independent recessives, each capable of bringing about either complete or a high degree of sterility in the male inflorescence. Meiosis occurs normally and the pollen grains are formed but before anthesis these grains abort. The anthers remain enclosed in the glumes. The tassel branches are thin and characteristically limp. In some cases a few anthers are extruded several days after anthers are exposed on normal sib plants, and rarely a small amount of pollen is released. This pollen is usually non-functional.

A similar type of pollen sterility in maize and other plants has been found to be conditioned by something outside the nucleus. RHOADES (1933) described the first case in maize. He found that it was transmitted only through the ovules, never through the pollen, and that in transmission it was independent of the chromosomes. All of the offspring of a sterile plant were likewise pollen sterile irrespective of the normal maize plants used to produce seed. RHOADES concluded that this condition was brought about by something in the cytoplasm.

Similar cases of cytoplasmic pollen sterility in maize have been found in other unrelated varieties and in five other genera: Linum, Nicotiana, Allium, Beta and Daucus.

In all types of cytoplasmic sterility pollen production varies widely from complete sterility to the production of functional pollen in varying amounts. GABELMAN (1949) has studied this type of sterility further and finds that it is dependent upon a discrete particle that is transmitted regularly from cell to cell during mitosis, but may be reduced in numbers but never eliminated entirely in some of the egg cells. These particles are never transmitted by the pollen and therefore are eliminated during meiosis from some of the sporocytes. Apparently one particle can prevent the functioning of a male germ cell. Since the particles are increased during mitosis but not during meiosis they have something in common with chromatin, dividing regularly when the chromosomes divide and not dividing when the chromosomes fail to divide at the reduction division.

Since there exists something, both within and without the nucleus, that is transmitted from generation to generation, each having similar results, it is necessary to distinguish between these inherited determiners. Genes in the

chromosomes can logically be called chromogenes. The determiners in the cytoplasm have been referred to as cytogenes or plasmagenes. Both terms carry the implication that the nuclear and extra nuclear factors are similar. Since there is evidence that they are similar in some respects the term plasmagene is used here.

Both the plasmagene and the chromogene for pollen abortion have been combined in the same plants. By crossing and backcrossing a white-seeded, cytoplasmic-sterile plant by using normal pollen from a heterozygous gene-sterile plant, also heterozygous for yellow-white endosperm color, it was possible to obtain on a cytoplasmic-sterile plant yellow seeds that were heterozygous gene-fertile and white seeds that were homozygous gene-sterile. These two lots of seed were grown separately.

The heterozygous gene-fertile, cyto-sterile plants showed a few extruded anthers and produced a small amount of pollen. The same amount of pollen production was shown by other crosses of these cytoplasmic-sterile plants where the gene for sterile pollen was not involved. These plants averaged 79.2 inches in height compared to 76.3 inches for the gene-sterile, cyto-sterile plants in an adjoining row. This small difference in height could be due to the slightly greater heterozygosity of the yellow seeded plants. The gene-sterile,cyto-sterile plants showed no anthers and no pollen was released. The two different types of genes when present together in the same plant seem to be without effect on each other. Each gene had the same effect on pollen production as it has when acting alone.

Variation in the production of normally functioning pollen grains is characteristic of all the cytoplasmic pollen-sterile plants so far studied. Material from three different sources has been grown and two additional sources have been reported. In the process of converting standard inbred lines used in the production of commercial hybrid seed corn, it has been found that some lines are relatively easy to sterilize and others are difficult. The usual procedure is to cross the fertile inbred line on to selected, completely pollen-sterile plants of approximately the same maturity. By repeated backcrossing with the same fertile inbred on to sterile plants that were selected as the nearest in type and time of flowering to the inbred used as the pollinator, it was possible to convert a fertile line into a similar sterile line after from three to five backcrossings. This is easily accomplished since all that is necessary is to put a complete chromosome set from one type into the cytoplasm of the sterile type. No chromosome crossing over is involved as in the case where a single gene is being transferred and recombined into a new chromosome set.

The complete control of the chromogenes on size and maturity is illustrated in a striking manner in a cross and backcross of a sterile late southern field corn line with a fertile, very early, sweet corn line. The late field corn plants grew over ten feet tall in Connecticut and flowered about 90 days after planting. The early sweet corn inbred by which it was pollinated grew about four feet high and flowered in 60 days. After three backcrosses the sterile version of the early line is practically identical in size, structural details, and in time of flowering, to the early fertile inbred. Similar results have been obtained in

other combinations by making the original cross and backcrosses reciprocally, except that the crosses on cytoplasmic fertile plants remain fertile.

In other combinations as well, backcrossed sterile lines are similar in every respect to their corresponding fertile lines except in pollen production. The two lots grow to the same height, flower at the same time, and have the same structural details in all parts of the plant, as shown in table 1. This is further proof that the cytoplasm has very little to do with the ordinary variations within species. This fact had been well established by the similarity in reciprocal crosses within the species. Both lines of evidence show that the ordinary varietal differences are brought about by chromogenic differences and that the cytoplasm is basically the same.

TABLE 1

A comparison of fertile and sterile maize plants having the same nuclear but differing in cytoplasmic constitution.

	FERTILE	STERILE	
5 Inbreds	72.3	70.1	Height of plant in inches
7 Single crosses	102.6	97.7	Height of plant in inches
7 Single crosses	58.5	58.3	Days to first silk
3 Crosses of three inbreds	111.7	108.9	Yield in bushels per acre
3 Crosses of four inbreds	123.9	119.0	Yield in bushels per acre

In general, the inbred lines that are weak in pollen production are easy to sterilize. The first generation crosses of such inbreds, on the plants used as sterilizers, are usually completely pollen-sterile and successive backcrossed generations also remain sterile. A few plants may extrude some anthers and a small amount of pollen may be produced. The inbred lines that are good pollen producers, the ones that are usually used as pollinators in the commercial production of hybrid seed corn, usually show much pollen production in the first crosses and successive backcrosses with the same sterilizer stocks.

When the anthers on these plants are stained with iodine solution some of the pollen grains appear to be fully formed and well stored with starch. GABELMAN (1949) has shown that the proportion of well filled and partially filled pollen grains varies widely in some progenies.

A few inbred lines and genetic stocks have the ability to restore almost complete normal pollen production to the first generation crosses of sterile by fertile. Pollen counts show up to 95 percent of normally well filled and dark staining pollen grains. For example, a sterile line of Illinois A inbred crossed by a linkage tester stock produced eleven plants of which four had 95 percent or more normal-appearing pollen grains, five plants with 30 to 50 percent normal-appearing pollen, and two plants with less than 5 percent. The same sterile inbred crossed by a dioecious male plant produced ten sterile and five fertile plants. Three of the fertile plants were self-fertilized and two produced well filled ears, and one was about one-fourth filled.

The pollen from partially fertile plants that is capable of producing seed

when used alone was applied in competition with normal plants. To test the ability to function in competition with normal plants, pollen was collected from yellow-seeded, partially fertile plants, used alone to test its ability to function, and then put on fresh silks of a white-seeded plant. At the same time pollen from other white-seeded plants was placed on the same flowers. Approximately equal quantities were applied. On seven ears produced in this way six yellow seeds were found among 1085 white seeds where equal numbers would be expected if both kinds of pollen were equally effective. See table 2.

TABLE 2

The effectiveness of pollen from partially-fertile, yellow-seeded, plants in competition with normal pollen from white-seeded plants on white-seeded (C20) plants.

SOURCE OF POLLEN	NUMBER OF WHITE SEEDS	NUMBER OF YELLOW SEEDS
A^{st}×Oh28	187	0
A^{st}×Oh28	86	0
A^{st}×Oh43	88	0
A^{st}×B164	232	0
A^{st}×B164	78	2
$C106^{st}$×Oh41	319	3
(Wf^{st}×Oh7)C107	95	1
	1085	6

Sterile C106 inbred crossed by normally fertile Ky21 produced two progenies of which all plants were normal in pollen production. A sterile single cross (Wf9 sterile×38-11) pollinated by the same Ky21 line produced an all fertile progeny. The same C106 sterile plant cross-fertilized by a first generation hybrid (Ky39×Ky21) produced 45 completely sterile and 22 completely fertile plants in five progenies. All of the fertile plants that were self-fertilized produced a full set of seed and an examination of the pollen showed 95 percent or more of normal appearing grains. This pollen has not been tested in competition with normal pollen.

The fertile plants of the cross sterile A by dioecious male and the linkage tester segregated in the second selfed generation into completely sterile and partially fertile plants with varying amounts of pollen production. The fertile plants from the crosses with Ky21 have not yet been grown but presumably will segregate since the combination of (Ky39×Ky21) F_2 on the sterile inbred segregates.

The pollen restoring ability of Ky21 was indicated in results obtained by JOSEPHSON and JENKINS (1948). They found that Ky21 gives normal pollen production in hybrids where Indiana 33–16 and Ky27 are used as seed parents. These two inbreds in other combinations where Ky21 was not used as pollinators were highly pollen sterile. Other inbreds that have the ability to restore pollen production, either completely or partially, are Texas 127C, Illinois Hy and Minnesota B164. A number of other lines commonly used in the commer-

cial production of hybrid seed corn have this ability to restore pollen in varying degrees.

All of the evidence shows clearly that there are chromogenes that have the ability to overcome the sterility usually exhibited in plants that have the sterile plasmagene. So far, no cytoplasmic sterile plant has been restored to complete fertility in all of the plants of the following generations. Whether the chromogenes in Ky21 and similar lines can do this when they are in the homozygous condition remains to be determined.

In a routine conversion of many commercial inbred lines to the sterile tassel condition it has been found that about half of these lines possess genes that restore pollen production to a moderate degree. Since the first outcrosses to these lines are usually variable in pollen production it shows that these lines, although highly inbred and carefully selected for uniformity, are nevertheless heterozygous with respect to these genes that normally have no visible effect and are not consciously selected for or against. This shows clearly that there is a considerable amount of enforced heterozygosity in maize inbreds as has been suspected from other evidence.

By testing individual plants of a normal fertile line for their inability to restore pollen production when crossed on to sterile plants, it is possible to select and establish sub-lines that maintain the sterile lines completely sterile. By a similar process it is possible to increase the ability of many lines to restore a high degree of pollen production in crosses with these sterile lines. It is easier to establish, and to maintain by continuous back crossing, completely sterile lines by using inbreds that are normally poor pollen producers and conversely the good pollen producers are more favorable material for restoring pollen production.

SUMMARY

1. Pollen abortion in maize and other plants is brought about by recessive genes in the chromosomes and also by extra-nuclear factors presumably propagated and transmitted in the cytoplasm.

2. These two kinds of inherited determiners are designated as chromogenes and plasmagenes.

3. Both plasmagenes and chromogenes are variable in their control of pollen production.

4. Other chromogenes have the ability to restore pollen production in variable amounts in plants that are completely cytoplasmically sterile in other combinations.

5. Plasmagenes that condition pollen sterility apparently have no other effect upon the growth and structural details of the plants.

6. Plasmagenes and chromogenes for pollen sterility when acting together in the same plants apparently have no effect on each other and are independent in their action.

7. Pollen produced on partially fertile plants with the plasmagene for sterility is less effective than normal pollen when in competition.

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THE INHERITANCE OF PHOTOPERIODIC RESPONSE AND TILLERING IN MAIZE-TEOSINTE HYBRIDS[1]

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Received February 1, 1950

THE importance of day length as a determining factor in the time of floral initiation in plants was first reported by GARNER and ALLARD (1920). Since then numerous investigations have been conducted on this phenomenon, and many plants have been classified into short-day, long-day and day-neutral types on the basis of their response to photoperiod. All known varieties of teosinte belong to the short-day group of plants, while North American maize varieties are little influenced by photoperiod, and may be classified as day-neutral types. The fact that fertile hybrids may be readily produced between maize and teosinte affords an excellent opportunity for a study of the inheritance of this short-day character in hybrids between the two species.

PHOTOPERIODISM

The exact physiological processes necessary to bring about the change from a vegetative to a flowering state are still unknown, despite the considerable information on this subject which has now been collected. Although several factors may influence the formation of flower primordia, it is generally recognized, as expressed by ROBERTS and STRUCKMEYER (1938), that the flowering state is a direct result of the internal condition of the plant, rather than a condition brought about by any particular external factor. However, of the external factors which ordinarily influence plants growing under natural conditions, photoperiod and temperature are undoubtedly of primary importance. THOMPSON (1940), in reviewing the work on relation of temperature to vegetative and reproductive development in plants, reports that the evidence shows the prevailing temperature may be a determining factor in photoperiodic response. The present concepts of the mechanisms of photoperiodism have been summarized by MURNEEK (1948), who states that the photoperiodic response is received through the leaves and transmitted to the meristem, and that light, in regard to both duration and intensity, is the activating agent. Various hypotheses to account for the differences in plant reaction to photoperiod have been advanced by CHOLODNY (1939), LANG and MELCHERS (1941), HAMNER (1942, 1944) and BORTHWICK, PARKER and HENDRICKS (1948), but so far there has been no conclusive evidence to warrant the acceptance of any particular proposal.

It has long been recognized that plant varieties or strains within a single species may differ to a considerable degree in their individual time of blooming.

[1] Presented as part of a doctoral thesis to the Faculty of the Graduate School of HARVARD UNIVERSITY in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

However, with the discovery that photoperiod could exert a pronounced influence on time of floral initiation, it soon became evident that varieties of sensitive species differed genetically in their response to photoperiod. GARNER and ALLARD (1930) and BORTHWICK and PARKER (1939) in soybeans, OWEN, CARSNER and STOUT (1940) in sugar beets, OLMSTEAD (1944) in side-oats grama and QUINBY and KARPER (1947) in sorghum have all reported a diversity in photoperiodic response, which was due to genetic variation among those strains within the species studied. Inheritance studies involving photoperiodic response, however, have been relatively few in number. GOODWIN (1944) obtained results from a study of the F_1 and F_2 generations involving three strains of the short-day species, *Solidago sempervirens* L., which indicated that the minimal number of gene pairs determining photoperiodic response approximated the haploid chromosome number (n=9). QUINBY and KARPER (1945) reported that three genes exist in the milo variety of grain sorghum which are influenced by photoperiod, and that they are capable of producing four maturity phenotypes under natural day-length conditions. LANG (1948) studied the inheritance of photoperiodic response in *Nicotiana tabacum* L. hybrids, and found that a single gene pair conditions the difference between Maryland-Mammoth, a short-day type, and Java, a day neutral form. However, the rather extreme range encountered in the day-neutral segregates of the F_2 population indicates that modifier genes also influence time of blooming in this group; or perhaps other factors in addition to photoperiod are operative.

It has been known for many years that teosinte is a member of the short-day group of plants, and previous studies have been conducted on the inheritance of this character in maize-teosinte hybrids. COLLINS and KEMPTON (1920) reported that no simple type of inheritance for days to anthesis was indicated by their data obtained from the study of a maize-teosinte F_2 hybrid population. LANGHAM (1940) studied the inheritance of photoperiodic response in crosses of Durango teosinte × maize, and interpreted the data as indicating a single gene was responsible for the short-day response, and that maize was dominant to teosinte for this character. MANGELSDORF and REEVES (1939) found no linkage of days to anthesis with marker genes on chromosomes 2, 4, 6 and 9 in a study of several backcross progenies of (Florida teosinte × maize) × maize. In a study of weak versus strong response to length of day in Nobogame teosinte × maize and Durango teosinte × maize F_2 hybrid populations, MANGELSDORF (1947) reported that in neither population was there an approach to a simple Mendelian ratio.

DESCRIPTION OF PRESENT STUDY

Previous investigations have definitely shown that teosinte varieties differ to a considerable degree in their response to photoperiod. Consequently, in order to determine the response of several varieties, as well as to obtain a rather adequate sample of the species, varieties from six different sources were used in this experiment. These varieties, with the exception of El Valle, have been designated by the name of the town near or in which they were collected.

As may be determined from the map shown in figure 1, giving places of origin of the different teosinte varieties, these varieties represent collections from a range of latitudes.

Three of the varieties are from Nobogame, Durango and Chalco respectively in Mexico, and three are of Guatemalan origin. One of the latter is from a collection obtained near San Antonio Huixta, another from a collection made near Jutiapa, and the third is of indefinite origin but resembles very much the commercial type used in the southern United States which is known as the

FIGURE 1.—Map of the southern United States, Mexico and Guatemala, showing original place of collection for each of the teosinte varieties used in the present study. The location of College Station, Texas, where the maize-teosinte hybrids were grown, is also given.

Florida variety. As this latter type, which has been designated as El Valle, is apparently similar to Florida teosinte in all respects, its place of origin has been shown as southern Guatemala, in accord with the conclusion by LONGLEY (1937) as to the source of the Florida variety.

In order to minimize the degree of variability within each of the teosinte varieties, strains with some degree of inbreeding were used whenever possible. S_1 strains of Nobogame, Durango and Jutiapa and an S_2 strain of Huixta were used in all crosses involving these teosintes. An individual open-pollinated plant served as a source of seed of the Chalco variety; the only seed available of the El Valle variety was that from an open-pollinated source.

Since the type of maize used also affects the appearance of the hybrids, a common multiple tester stock was used for making all crosses with the exception of those involving Jutiapa teosinte. The multiple tester was quite uniform as a result of inbreeding, and was homozygous for marker genes on nine of the ten chromosomes. The marker genes were *bm*$_2$, *lg*$_1$, *a*$_1$, *su*$_1$, *V*, *gl*$_1$, *j*$_1$, *wx* and

g_1. In addition, the marker gene *P* was introduced from the maize parent in some of the crosses. All of the teosinte varieties carried contrasted alleles of these genes.

A typical plant of the multiple tester maize parent is shown in figure 2. A plant of El Valle teosinte shown in figure 3 gives some idea of the difference in appearance of the two species under Texas conditions. The other Guatemalan teosintes resembled El Valle to a marked degree in plant growth, but the Mexican teosintes were considerably less vigorous and possessed fewer tillers. The F_1 hybrid of El Valle teosinte × maize is shown in figure 4. In the hybrids, also, as in the varieties, the Guatemalan teosintes were considerably more vigorous and produced a greater number of tillers than the Mexican varieties.

The study of all parental strains and hybrid populations was conducted at College Station, Texas (see figure 1) during the spring and summer of 1948, where the day length during the growing season approaches a maximum of approximately 14 hours. The seeds of each progeny were planted individually in 5-cm high paper cups in the greenhouse on April 17 to 19, and seedlings were transplanted to the field ten days later. Weather conditions in general were favorable from the time of planting until the latter part of July, although from the last week in July until mid-September the growth of all plants was definitely retarded by the hot, dry weather.

In addition to the parental progenies and F_1 hybrids, both F_2 populations and populations involving backcrosses to each parent were included in the experiment. An attempt was made to provide 400 plants in each F_2 population, 200 plants in each backcross, 15 of each of the teosinte parents and F_1's and 60 of the maize parent. Although seed shortages of certain crosses and unfavorable weather conditions after planting reduced the final number of plants used for study, approximately the desired number was available in most populations. The actual number of individuals in each population is given in table 1. In this table, as in all later tables, the variety of teosinte is used to designate the indicated generation of a cross involving that particular teosinte and maize.

Plants of all populations were examined continually during the period of pollen shedding, and the date of anthesis for each plant recorded. In actual practice it was frequently necessary to estimate the actual date of anthesis for

TABLE 1

Number of individuals in each population.

POPULATION	NOBOGAME	DURANGO	CHALCO	EL VALLE	HUIXTA	JUTIAPA
Teosinte parent	8	8	10	14	13	10
Teosinte-maize F_1	14	14	—	14	11	15
Maize parent	54	54	54	54	54	54
F_1×teosinte	179	188	197	148	76	114
F_1×maize	195	188	194	145	196	171
Teosinte-maize F_2	372	377	354	384	381	221

FIGURE 2.—Plant of the multiple tester maize parent.

FIGURE 3.—Plant of El Valle teosinte.

FIGURE 4.—Plant of the F_1 hybrid El Valle teosinte×maize.

plants blooming during the latter half of the summer. During this period, because of insufficient moisture and extremely hot weather, the anthers did not usually dehisce, and in many instances the tassels were formed but failed to emerge fully. There were some plants, particularly in the F_2 and teosinte backcross populations involving Huixta teosinte, that lived until September 1 or later and finally died without forming tassels. Their dates of anthesis were recorded as the time at which they died. While this procedure introduced some error, it seemed to be the best method to follow in the recording of the data. All plants so classified were of a very late group (135 days or more) for their population, so it is not felt that they were seriously misclassified.

Some difficulty was encountered in determining the exact date of anthesis for the teosinte parents, as all tended to flower during the latter part of the season when growing conditions were poor. Several plants of both the Nobogame and El Valle parents actually shed pollen, so the dates recorded for these varieties are accurate for the conditions encountered in 1948. Of the Jutiapa and Durango parental plants only two of each variety formed tassels, but as the duration of growth for several other plants indicated a similar period to flowering the estimates for those varieties should be relatively accurate. No plants of the Chalco and Huixta teosintes formed visible tassels, so their estimated dates of anthesis are based on the length of life of the latest plants of each variety. It is felt that the adverse growing conditions during the latter part of the season lengthened the time to flowering for late plants of all populations as well as the parental teosintes, and that under more favorable conditions the extremes in lateness would not have been quite so great.

In addition to the information obtained on date of anthesis for plants in all populations, data were also collected on number of tillers. Most teosinte varieties, and particularly those of Guatemalan origin, are characterized by a large number of tillers. On the other hand, most maize varieties tiller very little, and the strain used in this study exhibited no tendency whatsoever for tillering.

SEGREGATION OF MARKER GENES

Numerous studies have been conducted using marker genes in maize-teosinte crosses, and in most instances a normal segregation has occurred. However, KEMPTON (1924) in studying a hybrid of maize and Florida teosinte recovered only 12 percent of the *br* plants in the F_2 generation instead of the expected 25 percent. MANGELSDORF and REEVES (1939) reported a marked deficiency of plants segregating for the su_1 and *Tu* genes on chromosome 4, in a backcross population of (Florida teosinte × maize) × maize.

Segregation of marker genes in the backcrosses to maize and the F_2 populations which occurred in the present experiment are shown in tables 2–6. Chi-square values were calculated for goodness of fit to a 1:1 and 3:1 ratio for the backcross and F_2 progenies respectively, and P values for all genes are included in the tables. Although most of the genes behaved in a normal fashion, there were a few notable exceptions. The most striking deviation was found in the sugary segregates of the El Valle backcross and F_2 progenies. Of the seed on the backcross ears only 13.4 percent were sugary, while on the F_2 ears

TABLE 2

Segregation of marker genes in Nobogame teosinte-maize populations.

CHROMOSOME	GENE	$F_1 \times$ MAIZE			F_2		
		TEOS. GENE	MAIZE GENE	P VALUE	TEOS. GENE	MAIZE GENE	P VALUE
1	Bm_2	87	108	.15	272	100	.40
1	P	—	—	—	29	45	$<.01$
2	Lg_1	107	88	.25	283	89	$>.50$
3	A_1	85	110	.10	232	140	$<.01$
4	Su_1	149	180	.10	738	185	$<.01$
6	Y	152	177	.15	200	723	.02
7	Gl_1	89	106	.20	273	99	.25
8	J_1	100	95	$>.50$	319	53	$<.01$
9	Wx	165	164	$>.50$	683	240	.50
10	G_1	89	106	.20	288	84	.20

8.0 percent were sugary. These results agree with those of previous investigators in demonstrating a deficiency of fourth chromosome maize genes in hybrids with Florida teosinte, a fact which gives additional support to the assumption that the El Valle and Florida varieties are quite similar. Although the cause of this deficiency cannot be determined from this experiment, it seems reasonable to conclude that it is related to the sterility which is found in the F_1 hybrids. BEADLE (1932), MANGELSDORF and REEVES (1939) and O'MARA (1942) have all reported pollen sterility in hybrids of Florida teosinte and maize. The exact mechanism causing this sterility is unknown, although it seems rather certain, according to the results of previous experiments by ROGERS (unpublished), that some factor on chromosome 4 is primarily responsible for this sterility which occurs in hybrids of the southern Guatemalan teosintes with maize.

TABLE 3

Segregation of marker genes in Durango teosinte-maize populations.

CHROMOSOME	GENE	$F_1 \times$ MAIZE			F_2		
		TEOS. GENE	MAIZE GENE	P VALUE	TEOS. GENE	MAIZE GENE	P VALUE
1	Bm_2	101	87	.30	277	100	.50
1	P	29	63	$<.01$	46	112	.25
2	Lg_1	103	85	.20	270	107	.10
3	A_1	96	92	$>.50$	255	122	$<.01$
4	Su_1	148	132	.30	642	179	.04
6	Y	152	128	.15	226	595	.10
7	Gl_1	117	71	$<.01$	340	37	$<.01$
8	J_1	104	84	.15	275	102	.30
9	Wx	133	147	.40	622	199	$>.50$
10	G_1	116	72	$<.01$	284	93	$>.50$

TABLE 4

Segregation of marker genes in Chalco teosinte-maize populations.

CHROMOSOME	GENE	$F_1 \times$ MAIZE			F_2		
		TEOS. GENE	MAIZE GENE	P VALUE	TEOS. GENE	MAIZE GENE	P VALUE
1	*Bm*$_2$	86	108	.10	258	96	.35
1	*P*	39	68	$<.01$	54	100	$<.01$
2	*Lg*$_1$	92	102	.50	272	82	.40
3	*A*$_1$	111	83	.05	243	111	$<.01$
4	*Su*$_1$	174	136	.04	725	168	$<.01$
6	*Y*	115	123	$>.50$	246	647	.10
7	*Gl*$_1$	111	83	.05	274	80	.30
8	*J*$_1$	114	80	.02	267	84	$>.50$
9	*Wx*	157	153	$>.50$	674	219	$>.50$
10	*G*$_1$	107	87	.15	282	72	.05

There is a marked deficiency of glossy segregates in those populations involving Durango teosinte. In the maize backcross 37.8 percent glossy plants were recovered, while only 9.8 percent were obtained in the F_2 population. A similar deficiency of glossy plants occurred in the Huixta teosinte-maize F_2 population, as only 14.2 percent were obtained. However, in the (Huixta teosinte $\times$ maize) $\times$ maize population this gene pair segregated in a normal 1:1 ratio. In previous studies of Durango teosinte-maize hybrids involving marker genes on chromosome 7 no deficiencies of the maize genes have been reported in the segregating populations. This deficiency of chromosome 7 maize genes found in the populations studied in this experiment, therefore, is not typical of all maize-teosinte hybrids, and does not always occur even in crosses of maize with the same variety of teosinte.

TABLE 5

Segregation of marker genes in El Valle teosinte-maize populations.

CHROMOSOME	GENE	$F_1 \times$ MAIZE			F_2		
		TEOS. GENE	MAIZE GENE	P VALUE	TEOS. GENE	MAIZE GENE	P VALUE
1	*Bm*$_2$	74	71	$>.50$	277	107	.20
2	*Lg*$_1$	64	81	.15	286	98	$>.50$
3	*A*$_1$	91	54	$<.01$	289	95	$>.50$
4	*Su*$_1$	194	30	$<.01$	460	40	$<.01$
6	*Y*	75	149	$<.01$*	139	361	.15
7	*Gl*$_1$	76	69	$>.50$	278	106	.25
8	*J*$_1$	96	49	$<.01$	306	78	.04
9	*Wx*	128	96	.04	360	140	.10
10	*G*$_1$	82	63	.20	308	76	.02

* Not segregating in 1:1 ratio.

An excess of the double recessive segregates of the a_1 gene on the third chromosome occurred in several progenies. In all F_2 populations, with the exception of the El Valle teosinte-maize hybrid, significant departures from a 3:1 ratio were obtained. The same tendency for an excess of a_1a_1 plants occurred in the backcross populations of Nobogame and Huixta, although there was no such indication in the Durango or Chalco backcrosses. The remaining significant deviations are in all likelihood of a random nature, or have been caused through difficulties in classification. In particular, seeds homozygous for su_1 or y were extremely difficult to classify in the F_2 progenies. The deficiencies of j_1j_1 plants found in several populations were in all probability brought about

TABLE 6

Segregation of marker genes in Huixta teosinte-maize populations.

CHROMOSOME	GENE	$F_1 \times$ MAIZE			F_2		
		TEOS. GENE	MAIZE GENE	P VALUE	TEOS. GENE	MAIZE GENE	P VALUE
1	Bm_2	82	114	.02	290	91	>.50
1	P	—	—	—	42	113	.50
2	Lg_1	87	109	.10	278	103	.35
3	A_1	81	115	.02	261	120	<.01
4	Su_1	123	134	.50	679	185	.02
6	Y	107	150	<.01	257	607	<.01
7	Gl_1	95	101	>.50	327	54	<.01
8	J_1	93	103	>.50	316	65	<.01
9	Wx	120	137	.30	661	203	.40
10	G_1	116	80	.01	110	28	.20

through a failure to identify this character in every instance. As plants homozygous for g_1 are usually weak, the deficiencies of this character probably resulted from a loss of such plants at germination or in the early seedling stage.

RESPONSE TO PHOTOPERIODISM

Previous investigations have shown that all teosinte varieties are sensitive to photoperiod, although there is considerable variation among the different varieties in the strength of their response. The results of EMERSON (1924), LANGHAM (1940) and O'MARA (1942) show that the Mexican teosintes will bloom much earlier than the Guatemalan teosintes, when both are subjected to a 10-hour photoperiod. North American maize is rather insensitive to photoperiod, although EMERSON (1924) and LANGHAM (1940) found that late types of maize from the more southern latitudes respond to photoperiod. BRUNSON (cf. LANGHAM 1940) has also reported a late-flowering type of maize, *cz*, which has an indefinite vegetative growth under field conditions, but can be brought into flowering under short-day conditions. The maize stock used in this experiment is adapted to the northern United States, and represents an early type which is insensitive to photoperiod.

The mean number of days to anthesis of each population is shown in table 7, and a measure of variation for each population is given as the standard error of the mean. The maize parent bloomed in approximately 58 days, while even the earliest teosinte variety required more than 100 days. The Mexican teosintes as a group flowered much earlier than the Guatemalan teosintes. There is an increase in mean number of days to anthesis as the percentage of teosinte germplasm increases within any group of progenies involving a particular teosinte.

The F_1 hybrids of the Mexican teosintes bloomed at approximately the same time as the maize parent, indicating that maize is almost completely

TABLE 7

Mean number of days to anthesis of each population.

POPULATION	NOBOGAME	DURANGO	CHALCO	EL VALLE	HUIXTA	JUTIAPA
Maize parent	58.2±0.40	58.2±0.40	58.2±0.40	58.2±0.40	58.2±0.40	58.2±0.40
F_1×maize	57.0±0.32	58.2±0.41	61.5±0.45	71.6±1.06	67.6±0.50	66.6±0.75
Teosinte-maize F_1	60.9±1.10	64.9±0.72	—	102.0±3.29	106.2±5.25	126.7±2.84
Teosinte-maize F_2	65.4±0.46	70.9±0.52	74.7±0.59	112.5±1.51	131.2±1.49	115.5±2.08
F_1×teosinte	74.7±1.00	87.0±1.07	94.4±0.70	172.9±0.96	178.3±1.04	175.7±1.23
Teosinte parent	110.4	137.5	146.0*	199.6	207.0*	206.2

* Actual date of blooming estimated.

dominant to these teosintes in photoperiodic response. Although no seed of the Chalco teosinte-maize F_1 was available to permit inclusion of this hybrid in the experiment, previous observations indicate that the Chalco teosinte-maize hybrid blooms at approximately the same time as the Durango teosinte-maize hybrid. The Guatemalan teosinte-maize F_1 hybrids flowered somewhat later than the maize parent. Apparently maize is incompletely dominant to this group of teosintes, since the mean number of days to anthesis of each of these F_1 hybrids approached the maize parent more closely than it did the teosinte parent. The rather wide range of blooming dates of these F_1 plants suggests that they are quite easily affected by environmental conditions. The mean blooming date of each of the (teosinte×maize)×maize populations approached the mean blooming date of the maize parent. The F_2 hybrids on the average bloomed slightly later than the F_1 hybrids, with the exception of the Jutiapa teosinte-maize cross. The (teosinte×maize)×teosinte progenies involving the Mexican teosintes were somewhat intermediate between the F_1 and the teosinte parent, but the Guatemalan teosinte backcrosses rather closely approached the teosinte parent.

The length of the photoperiod at College Station during that part of the year in which the populations were grown is given in figure 5. The hours of sunshine possible are shown for number of days from planting, as well as for time of year, so that the length of the photoperiod at which plants of any population flowered may be readily determined. The frequency distributions for number of days to anthesis of each population are shown in figures 6–11. The plants have been grouped by 5-day intervals rather than by daily inter-

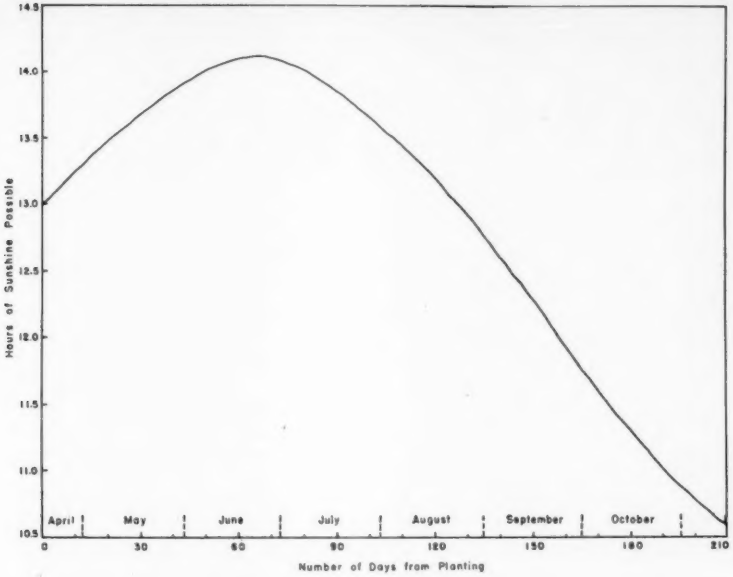

FIGURE 5.—The number of hours between sunrise and sunset during the growing season of the maize-teosinte hybrids at College Station, Texas.

vals in plotting their blooming dates. This grouping, however, has had no effect on the general appearance of the curves beyond smoothing out some of the irregularities.

These data show that the behavior of corresponding populations of each of the Mexican teosintes was quite similar. Likewise, the populations involving the Guatemalan teosintes, although differing from those of the Mexican teosintes, were quite alike in their behavior. Obviously the Mexican teosintes represent slight variations of a certain type of photoperiodic response, and the Guatemalan teosintes represent a group with a considerably stronger response.

The populations derived from Nobogame teosinte definitely show the weakest response to short day of any of the populations studied. The maize backcross progeny bloomed on the average slightly earlier than the maize parent, and both populations have a very pronounced mode at the same interval of 55–59 days. The F_2 mode is only slightly later than that of the maize backcross, occurring at the next interval of 60–64 days. Even the (teosinte×maize)×teosinte progeny exhibits a very weak response to length of day, most of the plants falling within the same range as those of the F_2 population. Apparently the day length of 13–14 hours, which prevailed during the growing season, is only slightly above the critical photoperiod for this particular variety of teosinte.

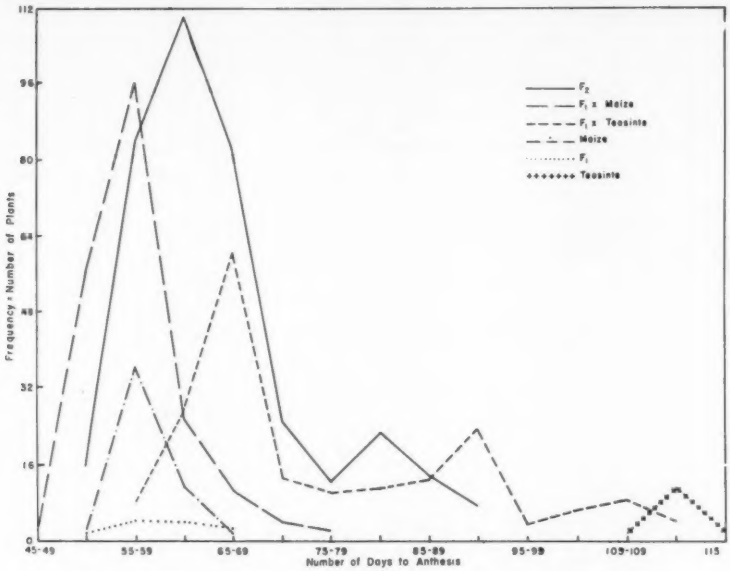

FIGURE 6.—Frequency distribution for number of days to anthesis in maize and all populations involving Nobogame teosinte.

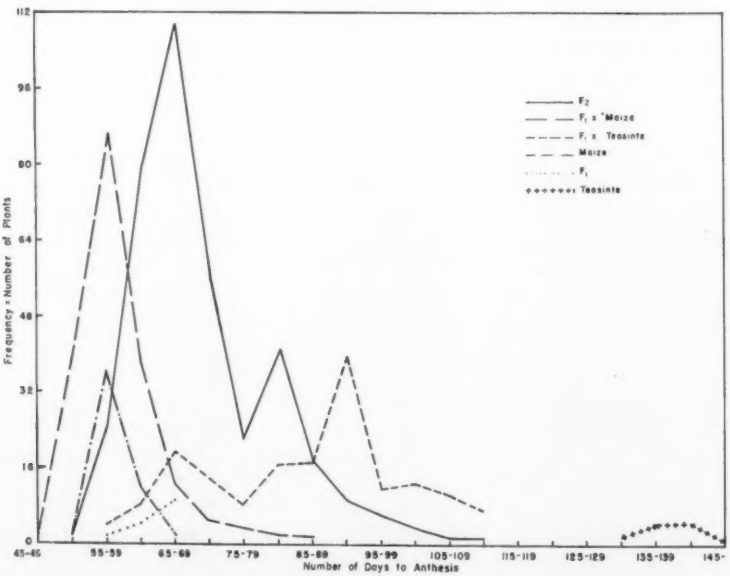

FIGURE 7.—Frequency distribution for number of days to anthesis in maize and all populations involving Durango teosinte.

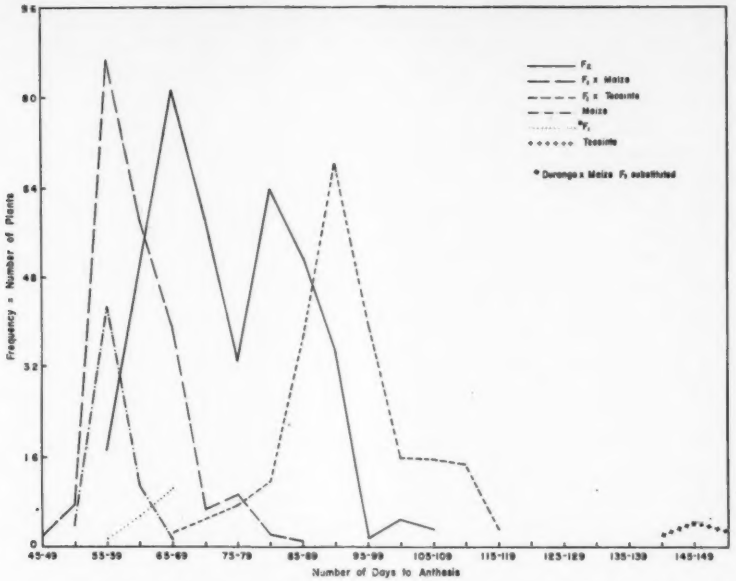

FIGURE 8.—Frequency distribution for number of days to anthesis in maize and all populations involving Chalco teosinte.

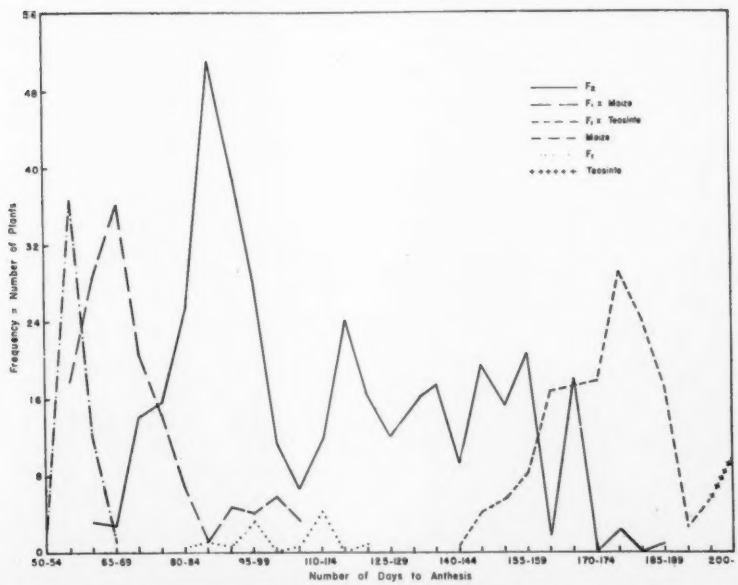

FIGURE 9.—Frequency distribution for number of days to anthesis in maize and all populations involving El Valle teosinte.

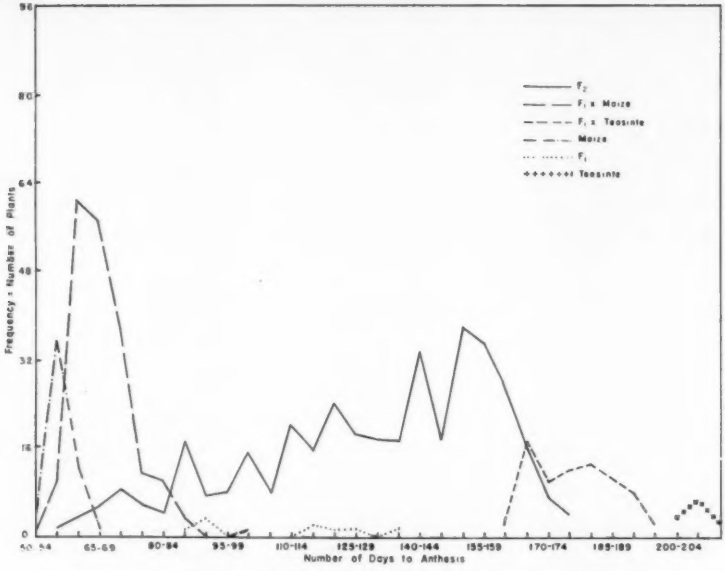

FIGURE 10.—Frequency distribution for number of days to anthesis in maize and all populations involving Huixta teosinte.

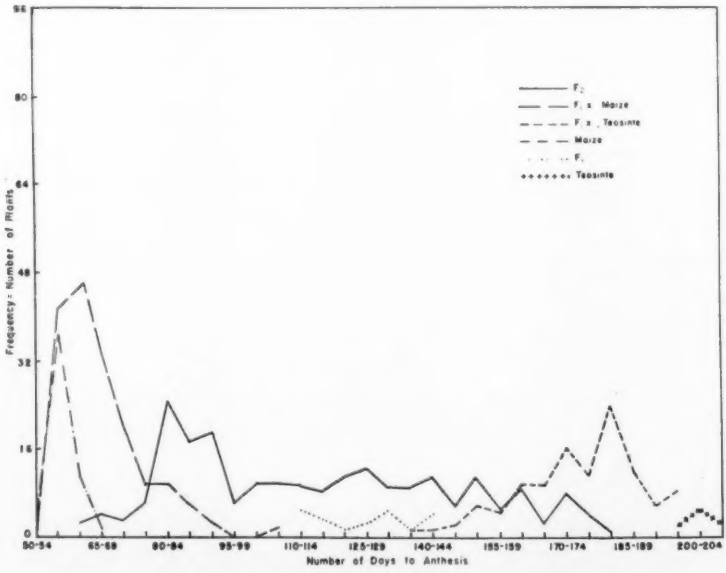

FIGURE 11.—Frequency distribution for number of days to anthesis in maize and all populations involving Jutiapa teosinte.

The Durango and Chalco hybrid populations responded in much the same manner under the conditions of this experiment. The maize backcross of each variety exhibits little variation, and the mode for each population occurs at 55–59 days. The F_2 hybrids of the two varieties also have the same mode of 65–69 days. However, the Chalco F_2 population is noticeably more variable and contains a higher proportion of late plants than the Durango F_2 population. The (teosinte×maize)×teosinte populations for both strains are somewhat intermediate between the F_1 and each parent. The Chalco population in particular has a definite mode approximately midway between the F_1 and Chalco teosinte. Although both the Durango and Chalco progenies approach the F_1 to a greater extent than they do the teosinte parent, this is probably caused to a large degree by the extreme growing conditions to which the teosinte parents were subjected.

The data clearly show that there is a definite sequence among the Mexican teosintes in the strength of their response to length of day. Nobogame shows the weakest response of the three teosintes, Chalco the strongest, and Durango is intermediate between the two. Segregation in all populations definitely shows, that for the photoperiod under which the experiment was conducted, the weak response to photoperiod of the maize parent is completely dominant, or almost so, to the strong response of the Mexican teosintes. There is, however, no evidence of simple Mendelian inheritance for this character in any population involving the Mexican teosintes.

The reaction of the Guatemalan teosintes in all progenies indicates that they possess a comparable genetic complex for photoperiodic response. Like the Mexican teosintes they exhibit a slightly variable response within this common behavior pattern, although the exact sequence among these varieties is not so clear. Considering all segregating populations, however, there seems little doubt but that the Huixta variety shows the strongest response of the Guatemalan teosintes.

The maize backcross progenies of all of these varieties strongly approach the maize parent, although a few plants overlap the range of the F_1 progenies. This distribution indicates dominance of the maize parent, although not of the almost complete type found in the Mexican teosinte crosses. The segregation in all of the Guatemalan teosinte-maize F_2 hybrids is quite pronounced, an extremely wide range occurring in all progenies. However, in the El Valle and Jutiapa populations the modal blooming date occurs near the blooming period of the maize parent, and in the Huixta progeny the majority of the plants approach the teosinte parent in time of flowering. The behavior of these F_2 populations, under the prevailing photoperiod, indicates that maize is partially dominant to the El Valle and Jutiapa varieties for photoperiodic response, but that the Huixta variety is partially dominant to maize for this character.

The distribution of blooming dates in the teosinte backcrosses is quite consistent for all of the Guatemalan varieties. These progenies show only a moderate degree of segregation, and approach the teosinte parent in each instance. This is a rather interesting occurrence in view of the fact that the maize backcross progenies of these same teosintes approach the maize parent

in time of flowering. These results rather definitely show, that regardless of the parent to which the backcross is made, the resulting progeny will display the flowering habits of the recurrent parent.

A rather striking abnormality noticed in plants of several segregating populations was a type of vegetative tassel. This unusual condition of the tassel was particularly noticeable in the Huixta teosinte-maize F_2 and the backcross of (Chalco teosinte×maize)×Chalco teosinte. One of the more pronounced types of vegetative tassel from the Chalco backcross progeny is illustrated in figure 12. As may be seen from the photograph this condition is characterized

FIGURE 12.—Vegetative tassel from the (Chalco teosinte×maize)×Chalco teosinte population.

by the development of vegetative rather than floral organs in the spikelet. A condition similar to this has been previously described in both maize and teosinte by REEVES and STANSEL (1940) and in maize by COLLINS (1909).

The occurrence of these vegetative tassels indicates that some plants may actually revert to a vegetative state after a certain degree of floral initiation. It seems reasonable to assume that for these plants the photoperiod was such as to induce originally formation of flowering primordia, but subsequent conditions altered the normal development of the staminate inflorescence to cause it to revert to a vegetative state. Perhaps such plants did not experience the required photoperiod for a sufficient period of time to permit normal development of the floral parts. LOEHWING (1939), in discussing the difference in photoperiod required for flowering and fruiting stages, suggests that the

photophase of plant development be divided into 1) an initial flowering phase and 2) a gametogenic phase. If they may be so divided, the behavior of the above mentioned plants may be explained by assuming that they received the correct photoperiod for floral initiation but not for the gametogenic phase.

NUMBER OF TILLERS

A difference in number of tillers is one of the characteristics which, like response to photoperiod, usually distinguishes maize from most varieties of teosinte. Since tillering is associated with photoperiodism in teosinte varieties, and since both are affected by the internal and environmental forces influencing the vegetative and reproductive stages, it seemed desirable to determine whether the same chromosomes are involved in the inheritance of tillering and photoperiodism.

Although there are types of maize which may tiller as profusely, or even more so, than some varieties of teosinte, most of the present-day varieties of field maize commonly grown in the United States evidence little tendency for tillering. A glance at the data in table 8 reveals that the maize stock used in this experiment produces no tillers, and that the teosinte varieties exhibit

TABLE 8

Mean number of tillers of each population.

POPULATION	NOBOGAME	DURANGO	CHALCO	EL VALLE	HUIXTA	JUTIAPA
Mazie parent	0	0	0	0	0	0
F_1×maize	1.89± .08	2.22± .13	0.91± .08	2.41± .16	1.60± .10	2.37± .11
Teosinte-maize F_1	2.00± .42	2.79± .39	—	4.07± .63	3.82± .40	3.93± .47
Teosinte-maize F_2	3.29± .10	3.22± .11	1.08± .06	4.08± .13	3.20± .10	5.38± .24
F_1×teosinte	4.51± .13	4.33± .16	1.28± .08	9.71± .41	10.13± .39	8.78± .53
Teosinte parent	5.38± .42	4.88± .48	1.10± .53	14.14± .98	10.92± .89	8.50± 1.06

varying degrees of tillering. All of the Guatemalan teosintes show a strong tendency for tillering, while the Durango and Nobogame varieties tiller moderately. The Chalco variety is quite similar to maize in this character, producing on the average slightly more than one tiller per plant. A consistent feature of all progenies involving a particular teosinte is an increase in mean number of tillers with an increase in percentage of teosinte germplasm.

The Nobogame and Durango teosintes behave very much alike in their effect on tiller number in the different hybrids with maize. The mean tiller number of the F_1 and F_2 populations of each variety is approximately intermediate between the two parents, and the mean of each backcross population falls between the mean of the F_1 hybrid and the recurrent parent. However, the means of these segregating populations do indicate some degree of dominance for the tillering habit of the teosinte parent. With the exception of the Nobogame teosinte-maize F_1, the F_1 and F_2 hybrids are closer to the teosinte parent than to the maize parent, and the backcross to teosinte approaches the

teosinte parent much closer than the backcross to maize approaches the maize parent. The degree of tillering in the Chalco hybrids, although much below that of the Nobogame and Durango hybrids, also indicates dominance for the tillering habit of the teosinte parent.

The Guatemalan teosintes show much the same type of behavior they exhibited for photoperiodic response. All varieties react in the same general manner in the different hybrids with maize, and from the behavior of the F_1 and F_2 progenies, the maize parent appears to be partially dominant to the teosinte parent. The mean number of tillers for each of the F_1 and F_2 populations including the Guatemalan teosintes, with the exception of the Jutiapa teosinte-maize F_2, is closer to the mean of the maize parent than to the mean of the teosinte parent.

The backcrosses to maize involving the Guatemalan varieties are somewhat intermediate between the F_1 and the maize parent, and exhibit a rather limited degree of tillering. The backcrosses to teosinte on the other hand tiller profusely, and approach the teosinte parent very closely in mean number of tillers. Here again, as in the response of these same hybrids to photoperiod, the backcross progeny displays the habit of the recurrent parent. This parallel behavior of the two characters is not surprising, since vegetative growth in plants, and hence production of tillers, is strongly affected by length of the growing period.

LINKAGE RELATIONS OF MARKER GENES WITH PHOTOPERIODIC RESPONSE AND TILLER NUMBER

A study of the linkage relations of marker genes with photoperiodic response and tiller number offers one of the most promising methods of determining the genetic nature of these two quantitative characters. Results of such a study not only give some idea of the number of factors that may be involved in the expression of such characters, but also serve to identify the chromosomes on which genes controlling the characters may occur. Linkage relations between the quantitative characters studied and the marker genes were determined for each maize backcross and F_2 population with the exception of those involving the Jutiapa variety. In testing for linkages between the qualitative marker genes and the quantitative teosinte characters, the group comparison method for classes of different size, as outlined by SNEDECOR (1946), was followed. With the use of this method a comparison was made between those segregates carrying the maize allele and those carrying the teosinte allele. The existence of linkage has been considered as established in those instances where the difference between the means of the two groups (maize allele versus teosinte allele) is significant, and the deviation is in the proper direction to indicate a linkage.

A summary of the linkage relations between photoperiodic response and the marker genes for each population is given in table 9, and the same data for tiller number are included in table 10. These tables contain the linkage

data for both the maize backcross and F_2 populations involving each of the teosinte varieties. Two symbols were used to denote the certainty of the linkages. Obviously more reliance may be placed on those relationships which have proven significant at the .01 level, than on those significant at only the .05 level. Strong deviations suggesting linkage, even though the differences were not quite significant at the .05 level, have likewise been indicated. Any significant deviations not due to linkage have also been included.

TABLE 9

Linkage relations of photoperiodic response with marker genes on nine chromosomes of maize, for each maize backcross and F_2 population involving different varieties of teosinte.

CHROM.	GENE	NOBOGAME		DURANGO		CHALCO		EL VALLE		HUIXTA	
		BC	F_2	BC	F_2	BC	F_2	BC	F_2	BC	F_2
1	*Bm*$_2$	−	S	−	−	−	+	−	S	I^a	−
1	*P*		−	−	−	−	+				−
2	*Lg*$_1$	−	−	−	−	−	−	++	−	+	+
3	*A*$_1$	S	I^a	−	−	−	S	−	−	−	−
4	*Su*$_1$	S	S	−	−	−	−	−	−	−	−
6	*V*	−	−	−	−	+	+	I	+	−	−
7	*Gl*$_1$	−	−	−	−	−	−	+	−	−	−
8	*J*$_1$	−	−	−	−	+	++	+	++	++	+
9	*Wx*	I^a	−	−	−	−	−	−	−	−	−
10	*G*$_1$	−	−	−	+	++	++	++	++	I	++

++ = Strong linkage, P < .01.
\+ = Linkage, P = .05–.01.
I = Indication of linkage.
− = Independent inheritance.
S = Strong deviation not due to linkage, P < .01.
I^a = Deviation not due to linkage, P = .05–.01.

The data conclusively show that genes controlling response to short day are located on chromosome 10 of all teosintes studied with the exception of Nobogame, and on chromosome 8 of all varieties except Nobogame and Durango. Chromosome 6 of Chalco and El Valle also carries genes influencing response to day length. Genes affecting this character definitely occur on chromosome 2 of Huixta, and perhaps on chromosome 2 and 7 of El Valle and chromosome 1 of Chalco. The linkages for all teosintes were usually consistent in both the maize backcross and F_2 progenies, although in some instances a linkage was obtained in only one of the two progenies of a particular variety. The results for short day response obtained in this experiment agree in part with those of MANGELSDORF (1947), who reported that chromosome 10 and perhaps chromosome 8 were involved in response to length of day.

It should also be noted that deviations in the wrong direction for linkage with the strong response for short day occur in several of the populations. This is particularly true in the Nobogame hybrids, where marker genes on chromosomes 3 and 4 show deviations in both the maize backcross and F_2

populations. The deviations for the marker gene on chromosome 4 may be accounted for by the slowness in growth of the *su*$_1$ plants, but there may actually be factors for earliness on chromosome 3 of Nobogame which produce what appears to be a reverse linkage. The occurrence of a significant deviation of this same type for the marker gene on chromosome 3 in the maize-Chalco F_2 lends additional support to this assumption that chromosome 3 of some varieties may possess earliness factors. As the other deviations in the wrong

TABLE 10

Linkage relations of tiller number with marker genes on nine chromosomes of maize, for each maize backcross and F_2 population involving different varieties of teosinte.

CHROM.	GENE	NOBOGAME		DURANGO		CHALCO		EL VALLE		HUIXTA	
		BC	F_2	BC	F_2	BC	F_2	BC	F_2	BC	F_2
1	*Bm*$_2$	+	+	++	+	+	+	++	++	++	++
1	*P*		−	−	−	−	−				−
2	*Lg*$_1$	+	++	++	++	++	−	++	+	++	++
3	*A*$_1$	−	−	−	−	−	+	−	−	−	−
4	*Su*$_1$	−	++	−	−	−	I^a	−	−	++	++
6	*Y*	−	−	+	+	−	−	−	−	−	−
7	*Gl*$_1$	++	++	−	++	++	−	++	−	++	++
8	*J*$_1$	−	++	−	I^a	−	−	−	−	−	−
9	*Wx*	+	−	−	−	−	−	−	−	−	−
10	*G*$_1$	−	++	−	−	−	−	−	−	−	−

++ = Strong linkage, P < .01.
\+ = Linkage, P = .05–.01.
− = Independent inheritance.
I^a = Deviation not due to linkage, P = .05–.01.

direction are those for the marker gene on chromosome 1 in the Nobogame F_2, El Valle F_2 and Huixta backcross, perhaps factors for earliness are also located on chromosome 1 of several teosinte varieties. The fact that these deviations are limited to a few chromosomes affords greater credence to the supposition that earliness factors do exist on chromosomes of the teosinte varieties.

Tiller number shows a linkage with the marker gene on chromosome 1 in all progenies, and a linkage with the marker gene on chromosome 2 in all progenies with the exception of the maize-Chalco F_2. Genes responsible for tillering are also indicated on chromosome 7 of all varieties, as linkages with the marker gene on this chromosome occur in either one or both of the progenies involving each particular teosinte. The data show that genes affecting tiller number occur on chromosomes 6 of Durango and 4 of Huixta, and possibly on chromosomes 4, 8, 9 and 10 of Nobogame and 2 of Chalco. The different teosintes are unusually consistent in the number of linkages shown by each variety, and also for the particular chromosomes involved in these linkages. These results indicate that each teosinte has approximately the same

number of factors affecting tiller number, with the possible exception of the Nobogame variety for which more are indicated.

Some caution must be exercised before definitely concluding that genes acting on tiller number are located on the chromosomes indicated by the linkage studies. Some of these apparent linkages may be the result of the direct action of the maize gene, as it is known that some recessive genes in maize actually influence the degree of tillering. This may be true in particular for the linkages found on chromosome 2, as it has been previously observed that plants with the liguleless character, which served as a marker for this chromosome, produce fewer tillers than normal liguled plants. The genes bm_2 and gl_1, which served as marker genes for chromosomes 1 and 7 respectively, are less likely to exert a direct effect on the tillering habit, and the significant relationships of these genes with tiller number probably represent actual linkages.

These data clearly show that genes governing each of the particular quantitative characters studied tend to be located on the same chromosomes in each of the varieties. Genes controlling photoperiodic response occur most frequently on chromosomes 8 and 10, and genes for tillering commonly occur on chromosomes 1, 2 and 7.

The average days to anthesis for each class with a different number of tillers were computed for each population, in order to determine whether any relationship existed between the two characters. No correlation was indicated from these calculations, however, as there was no consistent increase in days to anthesis with an increase in number of tillers.

DISCUSSION

Previous investigations on the physiological processes responsible for floral initiation in plants have shown that photoperiod and temperature are the primary factors which influence time of flowering. Individual varieties within a particular species (or closely related species) may differ primarily in their time of floral initiation, therefore, because of their response to either photoperiod or temperature. Differences due to photoperiodic response alone may be obscured by growing all varieties under a day length which is no longer a limiting factor in determining the time at which they flower. If length of photoperiod is thus removed as a limiting factor, and the differences still remain in time of flowering, it seems reasonable to conclude that these residual differences represent variation among varieties in thermal requirements. In other words, even under an inductive photoperiod some varieties take a longer period of time than others to attain the condition essential to floral initiation, and from a genetic standpoint might be regarded as carrying factors for lateness in addition to those affecting response to photoperiod. Previous studies in both maize and teosinte show that differences in time of flowering may still exist among varieties of each species when length of photoperiod is no longer a limiting factor.

Although no extensive experiments have been conducted on photoperiodic

response in maize varieties, the results available indicate that varieties of Central and South America are sensitive to photoperiod. MANGELSDORF (unpublished) has found that varieties from Guatemala, Costa Rica, Colombia and other Central and South American countries, which ordinarily do not flower until October when grown in the vicinity of Boston, are readily forced into flower with short-day treatment. North American varieties on the other hand are affected not at all, or at least very little, by photoperiod, and have apparently become adapted to a long photoperiod through a process of selection. According to HAMNER (1944), "If a given species contains any strains which are especially sensitive to photoperiod and can be classed without question into either the long-day or short-day group, then all of the other strains of the same species will tend to exhibit responses which would place them in the same class. For example, the varieties or strains of some species may be arranged in a graded series, at one end of which the plants are typical short-day plants and at the other end of which the plants are day-neutral or nearly so." As maize varieties apparently form a graded series such as that described above, it seems reasonable to conclude that maize is essentially a short-day species, although certain strains have been developed through a process of selection which are adapted to relatively long photoperiods.

All known varieties of teosinte behave as short-day plants, although the strength of response varies with the variety. Those varieties from Mexico consistently exhibit a weaker response than the Guatemalan varieties, and will also bloom much earlier even under 10-hour photoperiods. LANGHAM (1940) has shown that an early type of maize, such as the multiple gene tester used in the present study, blooms at approximately the same time as the Mexican teosintes when both are subjected to short-day treatment. Although no studies on short-day treatment were included in the present experiment, the behavior of the maize parent and teosinte varieties under short-day conditions in the greenhouse during the winter was similar to that reported by LANGHAM. The maize parent and Mexican teosintes bloomed at approximately the same time, and much earlier than the Guatemalan teosintes.

The inference from these results is that the difference between the maize parent and the Mexican teosinte varieties in time of flowering under long photoperiods must be due to those genes of the Mexican teosintes influencing response to photoperiod. In contrast, although the maize parent seems to differ from the Guatemalan teosintes primarily in those genes responsible for photoperiodic response, other factors which affect floral initiation under even the most favorable inductive photoperiod may also be involved. LANGHAM'S (1940) data on the short-day treatment of Huixta teosinte at different stages of growth throw some light on the factors which influence flowering in this variety. He found that plants in the seedling stage flowered in 101 days after short-day treatment was begun, while plants about six weeks old flowered after a short-day treatment of only 47 days. Apparently the Guatemalan varieties such as Huixta must attain a certain stage of development before short-day treatment is effective. These results suggest that the Guatemalan teosintes possess genes which prevent floral initiation until the plants attain

a certain stage of development, regardless of the length of photoperiod under which they are grown. Perhaps a thermal requirement must first be met before length of photoperiod becomes a limiting factor, and genes governing photoperiodic response become effective.

This study of the maize-teosinte hybrid populations throws some light on the functioning of the genes affecting response to photoperiod. The behavior of the Mexican teosinte-maize hybrids shows that under natural day length in Texas the maize parent is almost completely dominant to this group of teosintes in photoperiodic response. However, LANGHAM'S (1940) results indicate that the length of photoperiod may have an effect on the degree of dominance displayed by the maize parent, as in a study of Durango teosinte-maize hybrids at Ithaca, New York, he found that the F_1 hybrids bloomed somewhat later than the maize parent. This suggests that as the photoperiod increases maize is no longer completely dominant to teosinte, and indicates that the teosinte genes governing response to photoperiod are still active in the hybrid, and merely require a higher photoperiod than they do in teosinte to become effective.

The results obtained with the Mexican teosintes show that a very definite relationship exists between the strength of their response to photoperiod and the latitude at which they originated (see map, fig. 1). Also strength of response to photoperiod among these varieties is not completely correlated with other teosinte-like characteristics of the variety. The Chalco variety, which is definitely more maize-like in morphological characters than the Durango variety, exhibits a noticeably stronger response to photoperiod. As teosinte is constantly hybridizing with maize in Mexico, it appears that the maize with which each of the Mexican teosintes has been contaminated may be the controlling factor in their response. This photoperiodic response of the teosinte varieties is what might be expected if the response is primarily determined by maize varieties with which they hybridize, since MANGELSDORF (unpublished) has found that maize varieties also become more responsive to photoperiod as they approach the equator.

The Guatemalan teosinte-maize hybrids bloom much later on the average than the Mexican teosinte-maize hybrids, and the maize genes are not completely dominant to genes of the Guatemalan varieties in photoperiodic response. The wide distribution of segregates in the F_2 populations must be primarily due to those genes controlling photoperiodic response, since the teosinte parents themselves exhibit such a strong response under this same photoperiod. As previously suggested, however, the maize parent and the Guatemalan teosintes may differ by other factors which influence time of flowering, so there may be some segregation due to these factors. The changing photoperiod during the growing season may also have a pronounced effect on this distribution. A study of Guatemalan teosinte-maize hybrids under short-day conditions should provide additional information on the factors distinguishing this group of teosintes and maize in time of flowering.

In the backcross populations involving the Guatemalan teosintes very few of the segregates approach the F_1 hybrid, and the distribution of the segregates

indicates that the proportion of parental germplasm is the primary factor in determining time of flowering. In other words the maize genes are partially dominant in the backcross to maize, while the teosinte genes are partially dominant in the backcross to teosinte. This behavior illustrates a rather unique type of gene action, and cannot be accounted for by either the incomplete type of dominance frequently reported for quantitative characters, or by the pronounced dominance of one parent. The type of behavior occurring in these backcrosses is perhaps best described by the term "antithetical dominance," which was first suggested by ANDERSON and ERICKSON (1941) to explain those cases where the modifiers tend to favor one parental extreme or the other. In the present study the modifiers carried by the maize chromosomes apparently favor the maize genes which have a weak response to photoperiod, while modifiers carried by the teosinte chromosomes favor the teosinte genes which have a strong response. This behavior represents an actual reversal of dominance similar to that described by HARLAND (1932) for the normal versus crinkled character in Gossypium, where normal behaves as a dominant in *Gossypium barbadense* L., but is recessive when transferred to *Gossypium hirsutum* L. In these particular maize-teosinte hybrids the strong response of teosinte behaves as a dominant when teosinte germplasm is preponderant, but is more or less recessive when the genetic complex approaches that of maize. Although photoperiodic response is not actually controlled by a single gene, as is the crinkled character reported by HARLAND, the same general type of behavior apparently takes place for each of these characters in species hybrids.

Since photoperiodic response is a rather complex character, and its genetic nature is not completely understood, it becomes rather difficult to develop any precise explanation of the gene action in the two species for this character. The results obtained in the backcross populations might be considered as supporting the theory of dominance proposed by FISHER (1928, 1931), since it seems certain that dominance of the major genes controlling photoperiodic response is conditioned by modifying factors. However, it is difficult to see how this influence of the modifier complex on the dominance of other genes came about as postulated by FISHER: whereby the heterozygote gradually approached the wild type due to the action of modifying factors. Rather, it seems that there has been a selection of numerous genes, perhaps over a considerable period of time, which influence photoperiodic response under the conditions to which each of the species has been subjected. As this character is undoubtedly influenced by numerous genes in each species, this selection actually amounts to the development of a genetic complex which controls photoperiodic response. Therefore, as the genetic complex approaches that of either parent, the type of photoperiodic response also tends to approach that particular parent. As the multiple tester maize parent and the Guatemalan teosintes represent types which are adapted to extremely different conditions, it seems reasonable to conclude that the modifier genes would be selected in each type which favor blooming under the particular photoperiod of their native habitat.

The strong photoperiodic response exhibited by the Guatemalan varieties,

in contrast to that shown by the Mexican varieties, might well be expected because of the shorter days of their natural habitat. However, the response within the Guatemalan group of varieties does not strictly follow latitude, as the Huixta variety, which has the strongest response, occurs at a slightly higher latitude than the El Valle and Jutiapa varieties.

The linkage studies have shown that at least part of the genes responsible for photoperiodic response are found on the same chromosomes in the different varieties, particularly on chromosomes 8 and 10. As genes on these same chromosomes were found in both Mexican and Guatemalan teosintes, they must be genes which control response to photoperiod. It seems reasonable to conclude that these genes, because of their location on the same chromosomes of the different varieties, have had the same origin. Presumably these are also the genes having a major effect on photoperiodic response, although there are probably numerous genes on other chromosomes which have minor effects or which act as modifiers. Those teosinte varieties which exhibit the weaker responses now have either fewer or less effective genes governing response to photoperiod.

The behavior of the segregating populations indicates that the genetic complex of each variety has a pronounced effect on the action of these genes controlling photoperiodic response. Results obtained by HARLAND (1929, 1932, 1936) in studies of Gossypium hybrids tend to support this supposition, since he has proved experimentally that the expression of a particular gene may be affected by the modifier complex. He found that genes which segregated in a simple Mendelian ratio when tested within a species display a blending type of inheritance in species hybrids. In further studies he has shown that genes transferred from one species of Gossypium to another will have a quite different expression in the two species. For example, the gene for petal spot in *G. barbadense* L. produces a large spot, while in *G. hirsutum* L. the modifier complex causes the spot to be small. It seems quite probable that genes affecting photoperiodic response in certain of the teosinte varieties have lost a part of their effect through the introgression of maize germplasm which possesses modifiers curtailing their expression.

In summarizing these results, the evolution of photoperiodic response in the various teosintes is apparently influenced not only by the infiltration of maize germplasm, but also by the type of maize germplasm. Natural selection in all likelihood influences the development of a type of photoperiodic response best adapted to the particular habitat of each variety. The strong response to short day is not necessarily correlated with other teosinte-like characteristics of each of the varieties studied. In general, photoperiodic response is correlated with the geographical origin of each variety. This might be expected since a similar relationship exists between maize varieties and geographical origin. As this holds true for all except the Huixta variety, apparently this is the least maize-like of the teosinte varieties so far as photoperiodic response is concerned.

It is possible that response to short day does not represent a truly distinguishing characteristic between maize and teosinte, since there may be maize

varieties which exhibit a response to photoperiod as strong as that of teosinte. Response to photoperiod is certainly found in both species, and a considerable range of variation in this character probably occurs in both maize and teosinte. Further investigations on the nature of the photoperiodic response of maize varieties in Central and South America should provide information for a better comparison between the two species in this character.

The tillering habits of the various teosintes included in this study follow in general the same pattern which these varieties exhibit for photoperiodic response. The Guatemalan teosintes are quite similar in tillering behavior, and all show a much stronger tendency for tillering than the Mexican varieties. Within the group of Mexican varieties, however, Chalco teosinte, which exhibits the strongest response to photoperiod, is more maize-like than either Durango or Nobogame in the production of tillers. Degree of tillering in teosinte varieties, therefore, is not necessarily associated with strength of response to photoperiod.

Tillering habit within each of the teosinte varieties, as shown by the behavior of all segregating populations, exhibits no consistent relationship with photoperiodic response, since there is no correlation indicated between tiller number and days to anthesis in any of the populations. In addition, genes controlling tiller number in teosinte occur most frequently on chromosomes 1, 2 and 7, while genes for photoperiodic response are commonly found on chromosomes 8 and 10. The fact that genes affecting tiller number are usually found on the same chromosomes of the different varieties indicates a similar source of genes for this character. However, as these genes are not as effective in some varieties as in others, apparently their effect has been weakened by the introgression of maize germplasm.

SUMMARY

The inheritance of photoperiodic response and tillering was studied in several segregating populations of maize-teosinte hybrids. A multiple tester stock carrying marker genes on nine of the ten chromosomes served as the common maize parent in these progenies. Teosinte varieties from three sources each in Mexico and Guatemala were used in these hybrids to provide a representative sample of the species.

Each of the teosinte varieties exhibits a definite response to photoperiod, although the Guatemalan teosintes show a much stronger response than the Mexican teosintes. The weak response of the maize parent is almost completely dominant in all crosses with the Mexican teosintes, but the Guatemalan teosinte-maize crosses show an intermediate type of behavior indicating that neither parent is completely dominant. This differential response of the various teosinte varieties is probably due in a large part to the action of modifier genes which each carries. The behavior of the backcross populations involving the Guatemalan teosintes suggests that genes responsible for the time of flowering in each species are strongly favored by modifiers of their own genetic complex.

The distribution of plants in all segregating populations indicates that

several genes are responsible for the photoperiodic response of each of the teosinte varieties. The linkage studies show that at least part of the genes responsible for the photoperiodic response are found on the same chromosomes in the different varieties, particularly on chromosomes 8 and 10. Presumably these genes have had the same origin.

In general, photoperiodic response of the different teosinte varieties shows a definite relationship with the geographical origin of each variety. Since a similar relationship exists between photoperiodic response and geographical origin in maize varieties, it seems likely that the introgression of maize germplasm is an important factor in determining the photoperiodic response of the teosinte varieties.

Number of tillers is largely controlled by genes on the same chromosomes (1, 2, 7) of the different varieties, indicating a similar source of genes for this character. However, these genes are much more effective in the Guatemalan than in the Mexican teosintes, as their effect in the latter group of varieties has apparently been weakened by the introgression of maize germplasm.

Tillering habit and photoperiodic response exhibit no definite relationship in any of the segregating populations, indicating that these two characters are controlled by different genes which are not closely linked.

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THE INHERITANCE OF INFLORESCENCE CHARACTERS IN MAIZE-TEOSINTE HYBRIDS[1]

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THE realization near the end of the last century that a close relationship existed between maize and teosinte has resulted in an interest from that time to the present in the exact nature of the differences distinguishing the two species. The role that teosinte may have played in tne origin and evolution of maize has been given particular attention. Some investigators have suggested that maize originated directly from teosinte by a series of mutations, while others have postulated that it may have arisen through hybridization of teosinte with some unknown species of the Andropogoneae. Hypotheses have also been advanced that teosinte is not directly concerned in the origin of maize, and is perhaps a descendant from a common ancestral form or a recent hybrid of maize with the related genus Tripsacum. All investigators, however, are in agreement that maize and teosinte are closely related, and that they had a common source of germplasm in a fairly recent phase of their evolution. A more thorough discussion of these theories concerning the relationship of maize and teosinte may be found in the work of MANGELSDORF and REEVES (1939) and MANGELSDORF (1947).

The most striking characteristic which distinguishes maize and teosinte is the extreme difference in structure of the pistillate spike of the two species. Although there is variation among different types of pistillate spikes within each of the two species, no types in one species approach any of the types found in the other species. Maize and teosinte represent two strongly discontinuous classes for this particular characteristic. Of the differences between the pistillate spikes of the two species the primary ones are paired versus single spikelets, many-ranked versus two-ranked arrangement of the spikelets and inconspicuous soft glumes versus prominent horny glumes. Several studies have been previously conducted to determine the genetic nature of these differences and, although the results have not always been in complete agreement, the conclusion in most instances has been that several factors determine the inheritance of these characters.

COLLINS and KEMPTON (1920) were the first to make a detailed inheritance study of characters in maize-teosinte hybrids. They measured 33 plant characters in an F_2 population of a cross between Tom Thumb popcorn and Florida teosinte, but found none in which a strictly Mendelian inheritance occurred. The only approach to a simple Mendelian inheritance was found in the arrangement of the spikelets in the pistillate inflorescence; the double spikelets of maize appeared to be a simple dominant over the single spikelets of teo-

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sinte. KEMPTON (1924) in a further study of hybrids between Florida teosinte and maize determined the inheritance of the recessive maize characters crinkly, ramose and brachytic. In some instances there was a correlation between the quantitative characters distinguishing the two species and the qualitative marker genes. KEMPTON called attention to the blending of characters in the F_2 as further proof of the close relationship between maize and teosinte.

MANGELSDORF and REEVES (1939) in a study of the backcross (Florida teosinte×maize)×maize reported a strong linkage of inflorescence characters differentiating maize and teosinte with marker genes on chromosome 4. However, they found little evidence of linkage with marker genes on chromosomes 2 and 6, and only a slight evidence of linkage with a marker gene on chromosome 9. LANGHAM (1940) studied the inheritance of paired versus single spikelets and many-ranked ear versus two-ranked ear in Durango teosinte-maize hybrids and their segregating populations. He concluded that maize was dominant to teosinte for these characters, and that both were inherited in a simple Mendelian fashion. MANGELSDORF (1947) made a further study of the inheritance of several inflorescence characters differentiating the two species in F_2 populations of Nobogame teosinte-maize and Durango teosinte-maize hybrids. These included the characters earlier studied by LANGHAM (1940), as well as two quantitative characters "glume score" and "disarticulation score," which were both measured in a wholly empirical fashion. None of the characters segregated as simple Mendelian recessives and linkages were detected on several chromosomes for most of them. MANGELSDORF concluded that chromosomes 1, 3, 4, 8, 9 and 10 carry more than their proportionate share of the genes differentiating the two species.

The present study was undertaken to obtain further information on the inheritance of the inflorescence characters, and in addition to determine the chromosomes on which genes affecting these characters are located.

MATERIALS AND METHODS

The inheritance of the inflorescence characters was studied in several F_1, F_2 and maize backcross populations involving six different varieties of teosinte. These latter included the Nobogame, Durango and Chalco varieties from Mexico, and the El Valle, Huixta and Jutiapa varieties from Guatemala. The common maize parent, which was used in all crosses with the exception of those involving Jutiapa teosinte, was quite uniform as a result of inbreeding and carried homozygous marker genes on nine of the ten chromosomes. The marker genes were *bm*$_2$, *P*, *lg*$_1$, *a*$_1$, *su*$_1$, *Y*, *gl*$_1$, *j*$_1$, *wx* and *g*$_1$. Progenies used for this study were grown in the spring and summer of 1948 at College Station, Texas. A more complete discussion of this same material, as well as a description of the conditions under which the experimental material was grown, is given in a recent paper by ROGERS (1950). The same progenies used in this previously reported study on photoperiodic response were also utilized for this study of inflorescence characters.

The two-ranked pistillate inflorescence bearing single spikelets, which is

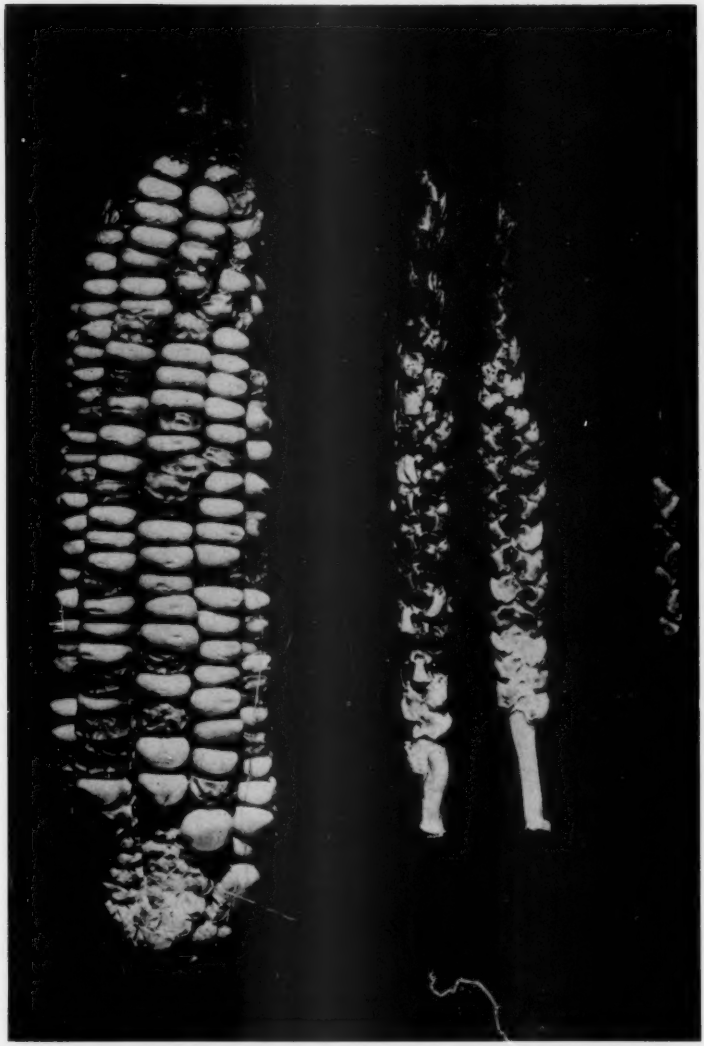

FIGURE 1.—From left to right. Pistillate spike of 1) maize parent, 2) Durango teosinte-maize F_1 hybrid, showing both many-ranked and two-ranked spikes, and 3) Durango teosinte.

characteristic of teosinte, offers a marked contrast to the many-ranked pistillate inflorescence bearing paired spikelets, which is found in maize. This difference is well illustrated in figure 1, where pistillate spikes of both parental types and the F_1 hybrid are shown. The spike illustrated is of the Durango variety of teosinte, and is rather typical of the pistillate spikes found in all varieties of teosinte. Examples of both many-ranked and two-ranked spikes of the F_1 hybrid are shown, since both types occur, even in crosses of maize

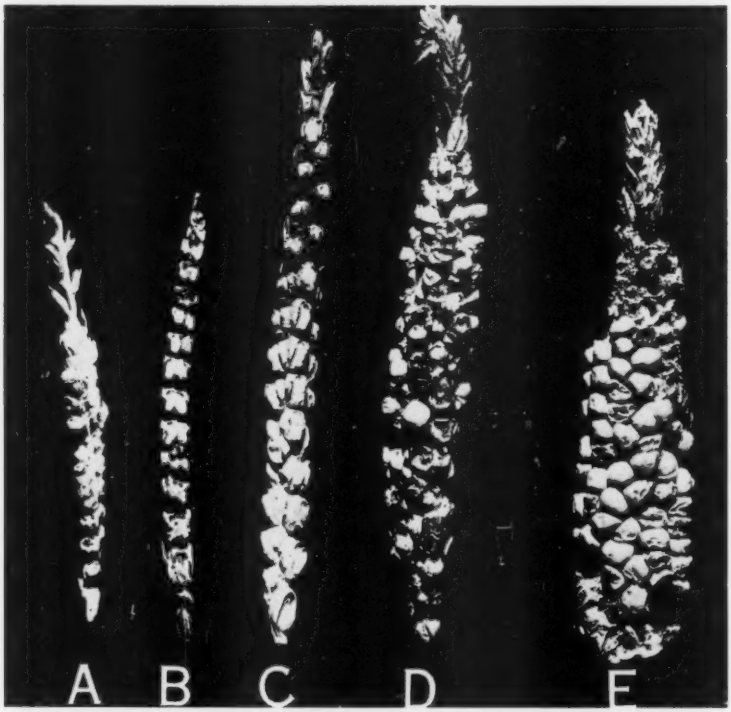

FIGURE 2.—Segregates from the Durango teosinte-maize F_2 population, showing range in type of pistillate spike. A. Two-ranked, single spikelets. B. Two-ranked, paired spikelets. C. Many-ranked, single spikelets. D. Many-ranked, paired spikelets, intermediate. E. Many-ranked, paired spikelets, resembling maize.

with a single variety of teosinte. Ordinarily in Guatemalan teosinte-maize F_1 hybrids a high proportion of the ears are two-ranked, while in Mexican teosinte-maize F_1 hybrids a high proportion of the ears are many-ranked. In all crosses studied in this experiment the F_1 hybrids have paired pistillate spikelets.

The pistillate inflorescences of both the Nobogame teosinte-maize and

FIGURE 3.—Segregates from the El Valle teosinte-maize F_2 population, showing many-ranked central spike (left) and two-ranked central spike (right).

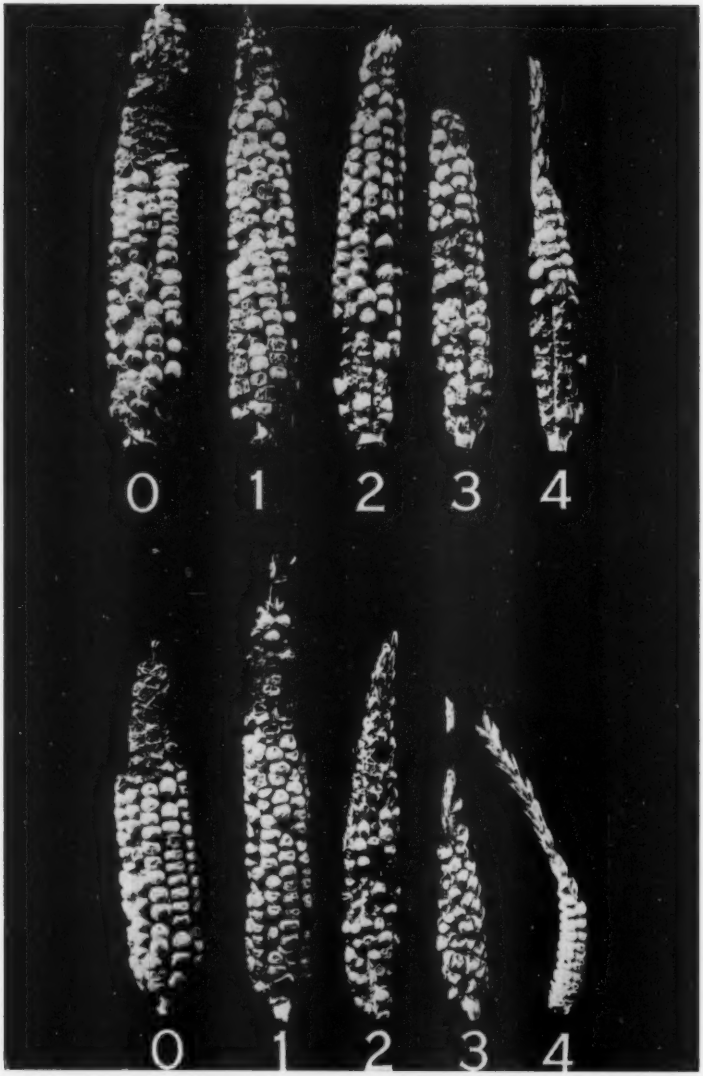

FIGURE 4.—Selected sample of each of the five ear grades from the cross (Nobogame teosinte ×maize)×maize (above), and the cross (El Valle teosinte×maize)×maize (below).

Durango teosinte-maize F_2 populations were classified on the basis of two-ranked versus many-ranked arrangement of the spikelets and paired spikelets versus single spikelets. A representative sample of ears from the maize-Durango F_2 population may be seen in figure 2, which shows the range from that type resembling the typical two-ranked teosinte spike to that resembling the many-ranked maize ear. An attempt was made to classify these characters in crosses of the Guatemalan teosintes, but so few of the plants produced ears that very little information was obtained. In the F_2 of El Valle teosinte×maize approximately one-fourth of the progeny produced pistillate spikes, and these were classified for the above mentioned characters. Practically no pistillate spikes were produced in either the maize-Huixta or maize-Jutiapa F_2 populations. In those F_2 progenies where analysis of the pistillate inflorescence was impossible, the central spike of the tassel was studied to obtain information on the inheritance of the two-ranked versus many-ranked character. For this character all teosinte varieties possess a two-ranked central spike, while maize has a many-ranked central spike. Segregates of both the many-ranked and two-ranked type of central spike from the maize-El Valle F_2 population are shown in figure 3.

The backcrosses to maize offered more favorable material for obtaining information on characters of the pistillate inflorescence than did the F_2 progenies, since practically all plants in each of the maize backcross populations produced ears. These progenies exhibited a range in ear type from that of the maize parent to that resembling the F_1. Although the F_1 varied somewhat in appearance depending upon the type of teosinte, it produced in all instances a very small spike quite unlike the maize parent. In order to determine whether linkage existed between any of the marker genes and quantitative factors affecting the ear, all backcross progenies were classified by ear grade. This was an arbitrary designation into five classes made according to the appearance of the ear, 0 resembling the maize parent and 4 the F_1. The intermediate grades 1, 2 and 3 represent a successive trend from the maize-like segregates to those like the F_1 hybrid. Selected samples of the various ear grades from the Nobogame and El Valle backcross populations are shown in figure 4. There was a considerable difference in appearance of the ears in the various backcrosses, and of those populations studied the one involving Nobogame teosinte had the most maize-like segregates, while the El Valle population was one of the least maize-like. This fact may be readily determined by an examination of the representative ears of the two populations.

CLASSIFICATION OF EAR AND TASSEL CHARACTERS

F_1 and F_2 Populations

The results from a classification of the F_1 hybrids given in table 1 show that the various teosintes differ in the strength of their expression on both ear and tassel characters. The varietal name of each teosinte is used in this table, as in all later tables, to designate the indicated generation of a cross involving that particular teosinte and maize. No data are shown for the Chalco teosinte-

maize F_1, since plants of this hybrid were not available for classification. The many-ranked pistillate and staminate inflorescence of maize is completely dominant in the F_1 cross with Nobogame teosinte. In the Durango hybrids both many-ranked and two-ranked pistillate spikes occur, although all tassels possess a many-ranked central spike. Apparently the genetic complex of the maize-Durango hybrid is of such an intermediate nature that either the maize or teosinte form of pistillate spike may result, depending perhaps on the environmental conditions at the time of development.

TABLE 1

Classification of characters in maize-teosinte F_1 populations.

CHARACTERS	NUMBER OF INDIVIDUALS				
	NOBOGAME	DURANGO	EL VALLE	HUIXTA	JUTIAPA
Many-ranked pistillate spike	14	5	0	0	0
Two-ranked pistillate spike	0	9	8	6	2
Total	14	14	8	6	2
Many-ranked central spike	14	15	10	4	14
Two-ranked central spike	0	0	4	7	1
Total	14	15	14	11	15
Paired pistillate spikelets	14	15	8	6	2
Single pistillate spikelets	0	0	0	0	0
Total	14	15	8	6	2

All of the Guatemalan teosinte-maize F_1 plants developing pistillate spikes produced the two-ranked type of the teosinte parent. However, both many-ranked and two-ranked central spikes occur in tassels of each of these same F_1 populations. The development of both types of central spikes in these F_1 hybrids involving a particular Guatemalan teosinte again illustrates the flexible nature of maize-teosinte crosses. The differential response of these Guatemalan teosinte-maize hybrids also indicates that the Huixta variety exerts the strongest effect on rank of the central spike. Of the three Guatemalan teosinte hybrids, the maize-Huixta F_1 has the highest proportion of two-ranked central spikes, followed in order by the maize-El Valle and maize-Jutiapa F_1 hybrids.

The maize-teosinte F_1 hybrids were consistent in their behavior for one character; all plants produced paired pistillate spikelets.

The data on ear and tassel characters of the F_2 populations, with the exception of the Chalco teosinte-maize progeny, are presented in table 2. In those populations classified for rank of pistillate spike, the frequency of two-ranked spikes is lowest in the Nobogame cross. The Durango hybrid has a somewhat higher proportion than the Nobogame hybrid, and the El Valle cross exceeds

both of the Mexican teosinte hybrids in frequency of two-ranked pistillate spikes. It is quite likely that the frequency of two-ranked spikes in the El Valle population would have been even higher, if a larger proportion of the later, more teosinte-like plants in the population had produced ears.

The same tendency exhibited in the F_1 hybrids for rank of the central spike persists in the F_2 progenies classified for this character. The greatest frequency of two-ranked central spikes is found in the Huixta hybrid, lower frequencies occurring in the El Valle and Jutiapa hybrids respectively. The failure of a

TABLE 2

Classification of characters in maize-teosinte F_2 populations.

CHARACTERS	NUMBER OF INDIVIDUALS				
	NOBOGAME	DURANGO	EL VALLE	HUIXTA	JUTIAPA
Many-ranked pistillate spike	223	130	25		
Two-ranked pistillate spike	103	184	70		
Total	326	314	95		
Many-ranked central spike			204	91	128
Two-ranked central spike			122	96	54
Total			326	187	182
Paired pistillate spikelets	248	188	56		
Single pistillate spikelets	78	126	39		
Total	326	314	95		

large number of the late plants to produce tassels probably resulted in a lower frequency of two-ranked central spikes in the Huixta population.

The paired pistillate spikelets of the maize parent predominate in all populations classified for this character. However, there is a definite increase in proportion of single spikelets from the Nobogame hybrid to the Durango hybrid, and from the Durango hybrid to the El Valle hybrid. Again, the frequency of teosinte-like segregates in the El Valle progeny in all likelihood would have been greater had more of the late plants produced ears.

The behavior of these ear and tassel characters in the F_1 hybrids, as well as their segregation in the F_2 populations, does not indicate a simple Mendelian type of inheritance. Although the segregation of paired versus single spikelets approximates a 3:1 ratio in the Nobogame teosinte-maize F_2, it cannot be assumed that the character is controlled by a single gene. Depending upon the variety of teosinte used in a hybrid with maize, almost any type of segregation may occur in the F_2. Apparently it is by chance that the segregation in the Nobogame hybrid is such that it approaches a simple Mendelian ratio.

The behavior of the characters for both pistillate and staminate spikes obviously varies with the variety of teosinte used in the cross. Results in both

the F_1 and F_2 populations are consistent in showing that fewer teosinte-like segregates for these characters occur in the Mexican teosinte-populations than in the Guatemalan. In a comparison between the two Mexican hybrids, it is at once apparent that the Durango variety exerts a more pronounced effect on the ear characters than does the Nobogame variety. Among the Guatemalan teosintes, the Huixta variety influences the ear and tassel characters in both F_1 and F_2 populations to a greater extent than do the other varieties studied. El Valle teosinte is in turn more effective than the Jutiapa variety in controlling characteristics of the ear and tassel.

Maize Backcross Populations

The results from a classification of the (teosinte×maize)× maize populations for ear grade and glume score are presented in table 3. As mentioned previously, these classes for both ear grade and glume score represent arbitrary

TABLE 3

Classification of characters in (maize×teosinte)×maize populations.

CHARACTER AND CLASS NUMBER	NUMBER OF INDIVIDUALS					
	NOBOGAME	DURANGO	CHALCO	EL VALLE	HUIXTA	JUTIAPA
Ear Grade						
0	11	8	4	5	12	3
1	42	19	33	12	29	16
2	100	50	77	46	77	67
3	40	80	62	54	53	44
4	2	28	11	10	11	14
Total	195	185	187	127	182	144
Glume score						
0	6	7	6	6	13	3
1	64	22	43	18	39	24
2	77	68	76	49	82	60
3	48	88	62	54	48	57
Total	195	185	187	127	182	144

groupings which have been made for the purpose of studying linkage relations of marker genes with these characters. The distribution of individuals in the various classes, therefore, does not represent an exact segregation that occurs for these characters. This grouping by classes, however, does furnish an index to the general nature of the segregates in a particular population. The populations with the higher frequencies in classes 0 and 1 for either character are more maize-like than those populations with lower frequencies in these classes.

An almost continuous range of ear types from one parental type to the other occurs in all of these maize backcross populations. Also there are very few segregates in any of the crosses that are approximate duplicates of the original

parental types. Therefore, ear grades 0 and 4 represent the two extremes of a population rather than the parental types, and grades 1, 2 and 3 include the various intermediate types. In a comparison of effects of the various teosintes on these characters, grade 1 is considered to include maize-like segregates and grade 3 to include F_1-like segregates. In the classification for glume score, the 0 class includes those segregates with a very light glume similar to that of the maize parent, and class 3 includes all segregates with a strongly developed horny glume. Class 1 approaches the maize type rather closely, and class 2 includes those ears with moderate glume development.

This study of the maize backcrosses again demonstrates that the various teosintes differ in their effect on morphological characters of the pistillate spike in hybrids with maize. Those crosses with the greatest frequency of F_1-like segregates for ear grade also show a high frequency of F_1-like segregates for glume score. In other words, there is a strong correlation between ear grade and glume score, and any teosinte variety exerting a strong effect on one also exerts a strong effect on the other.

Nobogame teosinte again exhibits the weakest effect of any of the teosinte varieties. The segregates of this cross are definitely more maize-like than those of the other crosses. However, it is interesting to note that the Huixta teosinte cross most closely approaches that of the Nobogame hybrid in frequency of maize-like segregates. This represents a definite reversal of the tendency displayed by this variety for those characters previously discussed, in which the Huixta variety exerted the most extreme effect of any of the teosinte varieties. The backcross population involving Chalco teosinte resembles that of the Huixta backcross in its proportion of maize-like segregates, indicating that these two varieties are somewhat similar in their effect on the two characters studied. The high frequency of F_1-like segregates in the Durango, El Valle and Jutiapa hybrids definitely show that these varieties have a more pronounced effect on ear characteristics of the backcross populations than do the other varieties studied.

The behavior of the different teosintes in their effect on characters studied in the backcross progenies differs somewhat from their behavior for the characters observed in the F_1 and F_2 populations. In this study of ear grade and glume score, Durango, one of the Mexican teosintes, produces a stronger effect than the Guatemalan variety, Huixta. However, for the inflorescence characters studied in the F_1 and F_2 progenies, there is a marked contrast in the behavior of the Mexican and Guatemalan teosintes, the latter group always exhibiting the most pronounced effect. It is felt that these results obtained in the maize backcross progenies represent a more accurate analysis of the different teosinte varieties in their effect on ear characters, since development of the pistillate inflorescence in both the F_1 and F_2 populations is definitely affected by response to photoperiod. In the maize backcross progenies these differences among the teosintes in photoperiodic response are minimized, and each variety has an opportunity to produce its effect on the inflorescence characters without appreciable influence from other factors.

The reaction of the Mexican teosintes in these maize backcross populations

shows conclusively that the Durango variety carries more effective factors influencing these ear characters than do the other Mexican varieties studied. Also, the rather high frequency of F_1-like segregates occurring in the Durango hybrid suggests that this variety actually possesses more factors than either the Nobogame or Chalco varieties. These results agree with those of MANGELSDORF (1947) on the comparative effect of Durango and Nobogame teosinte, as he also reports that Durango is the more potent of these two varieties. In fact, there is every indication that the Durango variety is just as potent as the El Valle and Jutiapa varieties in affecting ear structure and glume development in these maize backcrosses. Among the Guatemalan teosintes, it seems obvious that the Huixta variety possesses fewer and less effective factors influencing these characters than either the El Valle or Jutiapa varieties.

LINKAGE RELATIONS OF MARKER GENES WITH INFLORESCENCE CHARACTERS

A study of linkage relations between the marker genes and quantitative inflorescence characters serves to identify, at least in part, the chromosomes on which genes affecting these characters are located. Results of such a study also provide information as to whether the genes differentiating the inflorescences of maize and teosinte are scattered at random throughout the chromosomes, or occupy only a few sections of certain chromosomes.

A chi-square test for independent assortment was used to test for linkages between the marker genes and inflorescence characters. Comparisons involving each of the characters studied were made between those segregates carrying the maize allele and those carrying the teosinte allele, for each of the marker

TABLE 4

Linkage relations of ear grade and glume score with each other and with marker genes on nine chromosomes of maize, for each (maize×teosinte)×maize population.

CHROM.	CHARACTER	NOBOGAME		DURANGO		CHALCO		EL VALLE		HUIXTA	
		EG	GS	EG	GS	EG	GS	EG	GS	EG	GS
1	*Bm*$_2$	I	−	+	−	+	I	−	−	−	−
1	*P*			I	−	−	−				
2	*Lg*$_1$	−	−	+	−	−	−	+	−	−	−
3	*A*$_1$	+	−	−	−	−	−	+	I	I	I
4	*Sh*$_1$	++	++	++	++	++	++	−	−	++	+
6	*Y*	−	−	−	−	−	−	I	+	−	I^s
7	*Gl*$_1$	++	−	−	−	−	−	I	+	−	−
8	*J*$_1$	−	−	−	−	−	−	−	−	−	−
9	*Wx*	−	−	−	−	I	−	+	I	−	−
10	*G*$_1$	−	−	−	−	I^s	−	−	I	I	−
	EG		++		++		++		++		++

EG = Ear grade.
GS = Glume score.
++ = Strong linkage, P < .01.
− = Independent inheritance.
+ = Linkage, P = .05−.01.
I^s = Deviation not due to linkage, P = .05−.01.
I = Indication of linkage.

TABLE 5

Linkage relations of two-ranked spikes and paired spikelets with each other and with marker genes on nine chromosomes of maize, for each maize-teosinte F_2 population.

CHROM.	CHARACTER	NOBOGAME		DURANGO		EL VALLE			HUIXTA
		TR	PS	TR	PS	TR	TR[t]	PS	TR[t]
1	*Bm*$_2$	−	−	++	−	I	−	I	−
1	*P*	I[s]	−	−	−				−
2	*Lg*$_1$	−	−	++	−	I	−	I	−
3	*A*$_1$	++	−	−	+	++	I	I	−
4	*Su*$_1$	−	−	−	−	−	−	−	−
6	*Y*	+	−	−	−	I	−	−	−
7	*Gl*$_1$	−	+	−	+	−	−	−	−
8	*J*$_1$	−	−	−	−	I	++	I	+
9	*Wx*	−	−	−	−	−	I	I	−
10	*G*$_1$	−	−	−	−	−	+	I	−
	TR		−		−		++	+	
	TR[t]							++	

TR = Two-ranked pistillate spikes.
++ = Strong linkage, P < .01.
+ = Linkage, P = .05 − .01.
I = Indication of linkage.
TR[t] = Two-ranked central spike of tassel.
PS = Paired spikelets.
− = Independent inheritance.
I[s] = Deviation not due to linkage, P = .05 − .01.

genes individually. A linkage was considered as established if the distribution of individuals within the various classes was significantly different from that expected by chance, and the deviation was in the proper direction to indicate a linkage.

A summary of the linkage relations of the marker genes with the various inflorescence characters in the maize backcross and F_2 populations is shown in tables 4 and 5. No linkage data are available for the Jutiapa populations, since the multiple tester stock was not used as the maize parent in these crosses. The certainty of the linkage relationships between the marker genes and inflorescence characters has been designated for both the .05 and .01 levels of significance. Strong indications of linkage, as evidenced by the distribution of segregates in the various classes, have also been included in these tables for linkage relations. This latter type occurs most frequently for the ear grade and glume score characters, where a definite trend indicating linkage is sometimes exhibited, although it does not always prove to be statistically significant.

The most effective gene (or genes) acting upon ear structure occurs on chromosome 4, as ear grade shows a strong linkage with the marker gene on chromosome 4 in all but one of the progenies studied. This one exception is the progeny involving El Valle teosinte, and a satisfactory linkage test for chromosome 4 in this cross was impossible because of the extreme shortage of *su*$_1$ segregates. This pronounced effect on ear structure exhibited by chromosome 4 agrees with the results obtained by MANGELSDORF and REEVES (1939) and MANGELSDORF (1947) in studies of this same nature.

Several other genes also affect ear structure, but their location on the chromosomes varies with the variety of teosinte. Genes influencing ear structure are found on chromosome 1 of Durango, Chalco and possibly Nobogame teosintes, and on chromosome 3 of Nobogame, El Valle and possibly Huixta teosintes. Genes also occur on chromosome 2 of Durango and El Valle, on chromosome 9 of El Valle and possibly Chalco, on chromosome 7 of Nobogame and possibly El Valle, and perhaps on chromosome 6 of El Valle and chromosome 10 of Huixta.

The linkage data for glume score show that chromosome 4 of each teosinte, excluding the El Valle variety, carries the most effective gene (or genes) responsible for glume development. In fact, chromosomes 6 and 7 of El Valle teosinte are the only other chromosomes on which genes for this character definitely occur. Genes affecting glume development are also indicated on chromosome 1 of Chalco teosinte, chromosome 3 of Huixta teosinte and chromosomes 3, 9 and 10 of El Valle teosinte. These results on glume development agree only in part with those obtained by MANGELSDORF (1947), who reported genes for this character not only on chromosome 4 of Nobogame and Durango teosintes, but also on several other chromosomes of the two varieties.

Genes producing the two-ranked condition of the pistillate spike in teosinte are found on chromosomes 3 and 6 of Nobogame teosinte and on chromosomes 1 and 2 of Durango teosinte. LANGHAM's (1940) data also strongly indicate that a gene (or genes) governing the two-ranked condition of Durango teosinte occurs on chromosome 1. MANGELSDORF's (1947) results show that a situation similar to that reported for glume development prevails for the two-ranked condition, as genes for this latter character were also found to occur on several chromosomes of both Nobogame and Durango teosintes.

The data for two-ranked condition of the pistillate spike in the El Valle maize-hybrid show that genes occur on chromosome 3 and possibly on chromosomes 1, 2, 6 and 8 of this variety. The additional data obtained on the central spike of the tassel in the Guatemalan teosinte-maize hybrids show that genes controlling the two-ranked condition occur on chromosome 8 of El Valle and Huixta, on chromosome 10 of El Valle and possibly on chromosomes 3 and 9 of El Valle.

Genes responsible for single spikelets of the teosinte pistillate spike are found on chromosome 7 of Nobogame and Durango teosinte, on chromosome 3 of Durango teosinte and possibly on chromosomes 1, 2, 3, 8, 9 and 10 of El Valle teosinte. The failure to obtain statistically significant linkages in the maize-El Valle hybrid is probably due to the fact that only a small proportion of the individuals in this population could be classified for the single versus paired spikelet condition. LANGHAM's data (1940), which indicate a linkage of the single spikelet condition in Durango teosinte with the marker gene Lg_2 on chromosome 3, corroborate the present findings that a gene (or genes) for this character occurs on chromosome 3 of Durango teosinte. However, the present results do not agree with those of MANGELSDORF (1947), who reports that genes governing the single spikelet character are found on chromosomes 4

and 8 of Durango and on chromosome 4 and possibly chromosome 8 of Nobogame teosinte.

Ear structure and glume development are consistently correlated with each other in all populations studied. This strong correlation between the two characters indicates that the same genes or group of genes are largely responsible for both ear structure and glume development.

The linkage studies definitely show that several of the teosinte chromosomes may carry genes affecting the inflorescence characters which differentiate the two species. Although the particular chromosomes which are involved may vary somewhat among the different varieties, genes governing these characters are found on chromosome 4 of all varieties, with the possible exception of El Valle, and on chromosomes 1 and 3 of most varieties.

Genes responsible for the distinguishing ear characteristics of the two species are found on most of the chromosomes of El Valle teosinte, while only a few of the chromosomes of the other teosintes carry genes which strongly affect these characters. It is also significant that within this latter group the chromosomes exhibiting these strong effects vary to some extent with the variety. Genes with a strong effect on the ear characters occur on chromosome 1 of Durango and Chalco, on chromosome 3 of Nobogame and Huixta, on chromosome 7 of Nobogame and on chromosome 2 of Durango. These results strongly suggest, that for the ear characteristics, the El Valle variety differs from maize by genes distributed upon most of the chromosomes, while the other teosintes represent types which differ from maize by genes of a more localized nature on a few chromosomes.

DISCUSSION

As additional information is obtained from the study of maize-teosinte hybrids, it becomes evident that most of the ten chromosomes carry genes distinguishing the pistillate inflorescence of the two species. The data from the present experiment show that while the same chromosomes are often involved regardless of the teosinte variety studied, there are certain rather definite exceptions. The chromosomes carrying genes strongly affecting the ear characters sometimes differ from one variety to another.

These results, which show that genes affecting ear characters may occur on different chromosomes of the various teosintes, support the hypothesis that the more maize-like teosintes represent the original teosinte with a substitution of maize germplasm on various chromosomes. However, genes strongly affecting the ear characters occur on chromosome 4 of all varieties, which suggests that this chromosome in particular carries genes which are essential to development of the teosinte type of pistillate spike. The particular chromosomes on which the maize germplasm has been substituted may differ with the variety, since this introgression of maize germplasm has undoubtedly occurred at different locations. In fact, according to MANGELSDORF (1947) the transfer of maize genes to teosinte is still occurring at localities in Mexico today, and these teosintes are acquiring the characteristics of the surrounding corn. It is

probable that some of the Mexican varieties such as Nobogame have only a sufficient amount of teosinte germplasm to enable them to retain those characteristics necessary for survival in their present state of existence. The substitution of 3 or 4 sections of maize chromatin in some of these teosintes might actually result in the development of a maize-like type of pistillate inflorescence in these varieties.

The results of this experiment agree with previous findings by MANGELSDORF and REEVES (1939) and MANGELSDORF (1947) that the genes or groups of genes affecting structure of the pistillate spike tend to have similar effects. In the El Valle teosinte-maize hybrid, as indicated by the number of linkages of ear characters with the marker genes, it appears that the genes on different chromosomes have similar effects. There is no tendency for a pronounced linkage of these characters with marker genes on any chromosome as is so often the case in the other maize-teosinte hybrids, but rather an indication of linkage with the marker genes on several chromosomes. The more maize-like teosintes retain the essential characteristics of the teosinte spike, even though they possess genes on different chromosomes governing ear characters. This suggests that the particular chromosomes on which the maize germplasm has been substituted, with the exception of chromosome 4, are not too important, as long as a sufficient amount of the teosinte germplasm is retained to effect the teosinte type of pistillate spike. It seems that the El Valle variety functions more as a complete gene complex to produce the characteristic spike of teosinte, while the other teosintes studied are differentiated from maize by groups of genes at a limited number of locations on the chromosomes.

The strong correlation between ear grade and glume score in the present study strongly suggests that the same genes influence both ear structure and glume development. In previous studies of several ear characters, MANGELSDORF (1947) has also found that genes responsible for a particular character are frequently involved in some of the others. Apparently those genes or groups of genes responsible for the teosinte type of pistillate spike act in a general manner, rather than each gene or group of genes affecting only a particular character such as glume development.

Several differences exist among the teosinte varieties for the genes controlling ear characteristics. The El Valle variety carries genes affecting ear characters on most of the chromosomes, in contrast to the other varieties studied which carry genes responsible for these characters on only a few chromosomes. MANGELSDORF's (1947) report that the appearance of teosinte-like plants in a Florida teosinte-maize cross is more frequent than in crosses involving either Nobogame or Durango teosinte tends to substantiate these results. Since the El Valle and Florida teosintes are of southern Guatemalan origin, apparently the teosintes of southern Guatemala differ genetically from maize to a greater extent than do the teosintes of either northern Guatemala or Mexico so far as characteristics of the pistillate spike are concerned. The fact that each teosinte variety has retained the essential characteristics of the pistillate spike, although genes controlling the character may occur on different chromosomes of each variety, suggests that the more extreme form of teosinte, from which

these varieties have been derived, carried approximately the same kind of germplasm on different chromosomes.

In considering the present data, as well as those recently reported by ROGERS (1950) on the inheritance of photoperiodic response and tiller number in maize-teosinte hybrids, it becomes evident that the different teosinte varieties have retained varying amounts and types of germplasm affecting the various teosinte characters. None of the Mexican varieties possess the extreme response to short-day or high capacity for tillering found in the Guatemalan varieties. However, Durango teosinte has retained more effective genes controlling ear characters than the other Mexican or northern Guatemalan teosintes so far studied. The Huixta variety possesses an extreme response to photoperiod, but has apparently had some of the genes influencing the ear characters replaced by maize genes. Among the Mexican teosintes the Chalco variety shows the strongest response to photoperiod, yet exhibits one of the weakest effects of any of the varieties on ear characters, and has practically lost the ability to produce tillers. Nobogame represents a teosinte variety which has been so contaminated with maize that it probably has only the minimum amount of original teosinte germplasm necessary to enable it to retain those features of teosinte which clearly distinguish it from maize.

MANGELSDORF and REEVES (1939) have suggested that teosinte is a hybrid of maize and Tripsacum, and that teosinte is essentially maize on which four principal blocks of Tripsacum germplasm have been superimposed. The data from these recent experiments give no conclusive evidence as to the original relationship of maize and teosinte, nor do they render untenable the hypothesis that teosinte originated as a cross of Tripsacum and maize. The fact that genes affecting ear characters occur on most chromosomes of El Valle teosinte, as well as on most of the chromosomes of the Mexican teosintes when they are considered as a composite, indicates that if this hypothesis is correct, the Tripsacum germplasm is distributed throughout the chromosomes to a greater extent than originally suggested by MANGELSDORF and REEVES.

These results do emphasize the complex genetic differences between the two species, and lead to the conclusion that teosinte is not the progenitor of maize. At least it does not seem possible that maize could have originated from teosinte as the consequence of a small number of mutations. It does seem certain that in the original form of teosinte the genes distinguishing maize and teosinte were located on most of the ten chromosomes, and in the evolution of the present forms of teosinte each variety has retained some portion of the germplasm responsible for these distinguishing characteristics.

SUMMARY

Inflorescence characters were studied in several maize-teosinte hybrid populations involving a common multiple gene maize parent and six different varieties of teosinte. The maize parent carried marker genes on nine of the ten chromosomes. Teosinte varieties from three sources each in Mexico and Guatemala were used in the experiment.

None of the inflorescence characters studied exhibit a simple Mendelian

type of inheritance. The variety of teosinte has a pronounced effect on the proportion of teosinte-like segregates appearing in all of the segregating populations.

The linkage studies show that most of the ten chromosomes may carry genes distinguishing the pistillate inflorescence of maize and teosinte. Genes governing the ear characters are found on chromosome 4 of all varieties, with the possible exception of El Valle, and on chromosomes 1 and 3 of most varieties. However, other chromosomes exhibiting a strong effect on the ear characters may vary to some extent with the variety. Genes responsible for the teosinte type of pistillate spike occur on most of the chromosomes of El Valle teosinte, while only a few of the chromosomes of the other teosintes carry genes which strongly affect this character. The present results indicate that these genes or group of genes affecting structure of the pistillate spike tend to have similar effects.

The data now available from studies of maize-teosinte hybrids show that pronounced differences exist among the various teosintes in those characters which differentiate the two species. Since no single variety is least maize-like in all of the characters studied, it seems reasonable to conclude that all of the known teosintes represent varieties with some degree of admixture with maize, and they differ according to the type and amount of maize germplasm which each has acquired.

There is still no conclusive evidence on the original relationship of maize and teosinte, although the results of recent studies emphasize the complex genetic differences between the two species and tend to weaken the hypothesis that maize originated from teosinte.

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THE DETERMINATION OF LINKAGE INTENSITIES FROM F_2 AND F_3 GENETIC DATA INVOLVING CHROMOSOMAL INTERCHANGES IN BARLEY[1]

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THE calculation of linkage values between a qualitative factor pair and the point of interchange between two non-homologous chromosomes is relatively easy when backcross data are available. In crosses to a standard normal, partial sterility behaves as a dominant character with segregation for both the factor pair and sterility occurring in a 1:1 ratio. In a naturally self-pollinated species such as barley, the use of backcross procedures to obtain linkage intensities is not feasible which necessitates the utilization of segregating F_2 and F_3 data. In F_2 data sterility gives a ratio of 1 standard normal:2 semisterile:1 homozygous interchange normal. Since the normal classes are indistinguishable, a phenotypic ratio of 1:1 is expected for the sterility classification, while a completely dominant qualitative factor pair will give the expected 3:1 ratio. JOACHIM (1947) has shown that in such cases special formulas are necessary and has presented tables to facilitate the calculation of the linkage intensities by the product method and of the corresponding standard errors.

The separation of certain F_2 phenotypes into genotypes in F_3 rows will give additional information regarding linkage. In such cases it would be desirable to obtain a method for the determination of linkage from F_3 data and to combine this information with that obtained from the F_2. The purpose of this paper is to develop such a method for obtaining linkage values from different sources of segregating data involving interchanges and to devise a means of combining these sources of information. Data obtained from a study involving the segregation of partial sterility due to a chromosome interchange and certain qualitative factor pairs in barley will be used to illustrate this method. Although a partial sterility of approximately 25 percent is exhibited in barley, the term of semisterility will be used to designate individuals heterozygous for an interchange.

DERIVATION OF FORMULAS

Genetic Expectation

An F_1 individual heterozygous for an interchange (TN) and for a gene pair (Aa) will produce four types of viable gametes AT, aT, AN, and aN, where the symbols T and N are used to designate the interchange and the normal pairs of chromosomes respectively. Assuming that the recombination value p

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is the same in the two sexes, the gametic frequencies where *a* is carried by the parent homozygous for the interchange will be:

F_1 gametes	*AT*	*AN*	*aT*	*aN*
Gametic frequency	p/2	(1−p)/2	(1−p)/2	p/2

In the F_2 a *TN* zygote will give rise to a semisterile individual (SS) while plants from *TT* or *NN* zygotes will be normal (N) and indistinguishable. The F_2 phenotypes and the corresponding expected frequencies which may be tabulated from a Punnett square are:

F_2 Phenotype	*A*SS	*AN*	*a*SS	*a*N
Phenotypic frequency	$\frac{2-2p(1-p)}{4}$	$\frac{1+2p(1-p)}{4}$	$\frac{2p(1-p)}{4}$	$\frac{1-2p(1-p)}{4}$

Only the dominant *A* phenotypes will furnish further information regarding linkage in the F_3. In the separation of the F_2 phenotypic classes into genotypes the following frequencies are expected:

	AA	*Aa*	TOTAL
Semisteriles	$\frac{p(1-p)}{2}$	$\frac{1-2p(1-p)}{2}$	$\frac{1-p(1-p)}{2}$
Normal	$\frac{1-2p(1-p)}{4}$	$p(1-p)$	$\frac{1+2p(1-p)}{4}$
Total	1/4	1/2	3/4

The expression p(1-p) appears in all of the expected F_2 and F_3 frequencies. Thus, the expected frequencies remain the same whether the dominant gene enters the cross from the parent carrying the normal chromosomes or from the interchange parent, and coupling and repulsion phases can not be detected by phenotypic classification. Any formula derived from these expected frequencies must be applicable to either genetic cross.

Since all expected frequencies can be expressed in terms of p(1-p), recombination and non-recombination gametes must lose their identity as the result of F_2 and F_3 phenotypic classification of interchange data. This would mean that, essentially, the frequency of the recombination gamete can be determined only indirectly in formulas employing these expected frequencies. This limitation becomes apparent after a consideration of the zygotic frequencies tabulated with respect to the genotypes and the gametic origin as presented in table 1. All *AA* or *aa* normal zygotes and the *Aa* semisterile zygotes result from the union either of recombination or non-recombination gametes. However, the ratio of the recombination to the non-recombination union of gametes is such that each of the three genotypic classes reduces to terms of the product of the respective gametic frequencies. The *Aa* normal and the *AA* or *aa* semisterile zygotes result only from the union of a recombination gamete × non-recombination gamete. Thus, the frequency of the recombination gametes can not be studied directly; rather the parameter which can be measured directly is the product of the two respective frequencies, p(1-p).

Further, as a result of this limitation, no information could exist concerning the recombination gametes that would be entirely free from confounding by

the non-recombination gametes. At 50 percent recombination (table 1), recombination gametes are completely confounded with non-recombination gametes and little if any information regarding their identity is available. This has been illustrated by JOACHIM (1947) in her table 3 which shows that no information is available at $p=.5$, as computed by FISHER's general formula for this quantity. Recombination values other than .5 are reflected indirectly by deviations from a 3:1 ratio of *A* to *a* phenotypes in both the F_2 normal and semisterile classes and by the deviation of the *Aa* and *AA* genotypes from a 2:1 ratio. However, since the recombination and non-recombination values are not genetically separable, the deviation must be considered as a function of both.

TABLE 1

F_2 genotypic frequencies in normal and semisterile classes in relation to gametic origin.

PERCENT RECOMBINATION	GAMETES* COMBINED	NORMAL			SEMISTERILE		
		AA	*Aa*	*aa*	*AA*	*Aa*	*aa*
p	N×N	$(1-p)^2/4$	—	$(1-p)^2/4$	—	$(1-p)^2/2$	—
	N×R	—	$p(1-p)$	—	$p(1-p)/2$	—	$p(1-p)/2$
	R×R	$p^2/4$	—	$p^2/4$	—	$p^2/2$	—
50	N×N	.06	—	.06	—	.12	—
	N×R	—	.25	—	.12	—	.12
	R×R	.06	—	.06	—	.12	—
0	N×N	.25	—	.25	—	.50	—

*N = Non-recombination and R = recombination gametes.

These limitations in the measurement of p and in the estimation of the sampling variance of this quantity suggest the transformation, $x=p(1-p)$, for use with F_2 and F_3 interchange data. In this type of data x can be measured directly and an estimation of the sampling variance of x can be made for all recombination values. Thus, in the critical range where p approaches .5 and where little if any information concerning the identity of the recombination and non-recombination gametes is available, information does exist concerning the product of these two frequencies. This information based on x is available and can be used in adapting workable formulas for the combination of data from various sources by the method of scoring developed by FISHER (1946) and adapted for plant material by KRAMER and BURNHAM (1947).

Maximum Likelihood Formulas in Terms of x

With the substitution, $x=p(1-p)$, the expected phenotypic frequencies and the genotypic frequencies as determined by F_3 progeny rows can be expressed in terms of x. These expected frequencies are given in table 2 together with the observed numbers expressed as the quantities a to h. By the application of the method of maximum likelihood to these frequencies, formulas can be de-

TABLE 2

F_2 phenotypic and genotypic frequencies with the corresponding observed numbers in terms of x where $x=p(1-p)$.

CLASSIFICATION	F_2 PHENOTYPES			F_2 GENOTYPES IN THE A−CLASS		
	A−	*aa*	TOTAL	*AA*	*Aa*	TOTAL
Semisterile						
Expected frequencies	$(1-x)/2$	$x/2$	1/2	$x/2$	$(1-2x)/2$	$(1-x)/2$
Observed numbers	a	c		e	f	
Normal						
Expected frequencies	$(1+2x)/4$	$(1-2x)/4$	1/2	$(1-2x)/4$	x	$(1+2x)/4$
Observed numbers	b	d		g	h	
Total	3/4	1/4	1	1/4	1/2	3/4

veloped for the determination of linkage in any type of F_2 or F_3 interchange data. The basic concepts of this method of estimation have been reviewed and illustrated by MATHER (1946, pp. 203–208). The maximum likelihood formulas adapted for various types of F_2 and F_3 data are presented in column 2 of table 3.

TABLE 3

Maximum likelihood formulas for determination of x where $x=p(1-p)$ and formulas for the amount of information based on an estimate of x.

SOURCE OF DATA	MAXIMUM LIKELIHOOD FORMULAS	AMOUNT OF INFORMATION (i_x) PER INDIVIDUAL OR F_2 LINE
F_2, four classes	$-\frac{a}{1-x}+\frac{2b}{1+2x}+\frac{c}{x}-\frac{2d}{1-2x}$	$\frac{1}{2x(1-x)}+\frac{2}{1-4x^2}$
F_2, two classes due to recessive lethal	$-\frac{a}{1-x}+\frac{2b}{1+2x}$	$\frac{2}{(1-x)(1+2x)}$
F_2, six classes due to incomplete dominance	$\frac{c+e+h}{x}-\frac{2(d+f+g)}{1-2x}$	$\frac{2}{x(1-2x)}$
F_3, from semisterile dominant F_2's	$\frac{e}{x}-\frac{2f}{1-2x}+\frac{e+f}{1-x}$	$\frac{1}{(1-x)^2(x)(1-2x)}$
F_3, from normal dominant F_2's	$2\left(-\frac{g}{1-2x}+\frac{h}{2x}-\frac{g+h}{1+2x}\right)$	$\frac{4}{(1+2x)^2(x)(1-2x)}$

By setting the appropriate maximum likelihood formula equal to zero, the value of x which best fits the observed data is obtained, and this value of x would be the maximum likelihood estimate for the respective data. However, for any other value of x, the expression is not zero; it has a value which FISHER terms a score. This score becomes the basis for the combining of genetic data and for the estimating of linkage intensities by the method of scoring. Thus,

the maximum likelihood formulas in table 3 become the formulas for the scores (c_x) applicable to the estimation of x.

The Amount of Information

The total amount of information concerning linkage which is available in a body of genetic data depends upon the size of the sample N and an intrinsic portion i. This portion i, which depends on the value of the estimate, is referred to as the amount of information contributed by each individual or, in the case of F_2 data, by each row to the estimate of the parameter. Since x is the linkage function being estimated, the amount of information i_x may be determined by

$$i_x = S\frac{1}{m}\left(\frac{dm}{dx}\right)^2 \qquad (A)$$

where m is the expected proportion of the total in a class, dm/dx is the derivative of m with respect to x and S denotes summation over all classes (FISHER 1938, pp. 25–34). The general formulas for the determination of i_x are presented in column 3 of table 3. These values when multiplied by N, the number of individuals classified, give the total amount of information I_x furnished by each body of data.

Combining Data from Different Sources

The steps in analyzing linkage data from different sources include (1) testing the hypothesis of independent assortment between the characters being studied, (2) determining the homogeneity of various sources of data, and (3) if linkage is present, calculating a recombination value and its standard error which best fits all available data. FISHER (1946) presented the method of scoring for analyzing different sources of genetic data. Based on the transformation $x=p(1-p)$, formulas have been adapted for the method of scoring segregating data involving interchanges.

For testing independence between a factor pair and the interchange break, a convenient expression for χ^2 is given by

$$\chi^2 = \frac{c_x^2}{I_x} \qquad (B)$$

with one degree of freedom. The score c_x and the total amount of information I_x are both computed for the value of $x=.25$. The χ^2 test for homogeneity where there are n sources of data to be pooled is

$$\chi^2 = S\left(\frac{c_x^2}{I_x}\right) - \frac{(Sc_x)^2}{SI_x} \qquad (C)$$

with $n-1$ degrees of freedom and with summation over the n sources of data. An estimate of a combined x value for all data is

$$x = x' + \frac{Sc_x}{SI_x} \qquad (D)$$

where x′ represents the selected value for x and where the scores and the information again are summed over the n sources of data. Since these three basic equations are first considered for x=.25 for independence (the value of x taken for p=.5), the maximum likelihood formulas and the formulas for the amount of information i_x from table 3 are expressed for the constant x=.25 and are presented in columns 2 and 3, respectively, of table 4. The formulas in this table become the bases for the preliminary steps in the analysis of such genetic data.

TABLE 4

Formulas for the scores and for the amount of information when x=.25 for application to χ^2 for independence between the interchange break and a factor pair.

SOURCE OF DATA	FORMULAS FOR SCORES (c_x) AT x=.25	INFORMATION PER F_2 PLANT OR F_3 LINE (i_x) AT x=.25
F_2, four classes	$\frac{4}{3}(b+3c-a-3d)$	$\frac{16}{3}$
F_2, two classes due to recessive letha	$\frac{4}{3}(b-a)$	$\frac{16}{9}$
F_2, six classes due to incomplete dominance	$4(c+e+h-d-f-g)$	16
F_3, from semisterile dominant F_2's	$\frac{8}{3}(2e-f)$	$\frac{128}{9}$
F_3, from normal dominant F_2's	$\frac{8}{3}(h-2g)$	$\frac{128}{9}$

The first estimate of an average x value is generally not sufficiently close to the true combined x, but it may be substituted into the original maximum likelihood formulas in table 3 to obtain a second series of scores and information. These values would be substituted into the preceding equations to obtain a second estimate of x. The procedure is repeated until the estimated average value becomes constant.

The relationships presented have been proved by FISHER for ordinary linkage experiments. These relationships also will hold for the x transformation as adapted for interchange data. Further, after these three basic equations for the method of scoring are examined, the necessity of the x transformation for a workable method of scoring becomes apparent. It has been noted that no information exists concerning the estimate of the recombination value at p=.5. Since i_p is zero for this critical value of p, I_p also would be zero and the method of scoring based on an estimate of p can not be used. The condition is obviated by the use of x, the information i_x based on x being real and finite for this range of p (table 4).

Conversion to Recombination Values

After the appropriate x values have been obtained, they may be converted readily to the recombination value p. Since $x=p(1-p)$,

$$p = \frac{1 - \sqrt{1 - 4x}}{2}. \qquad (E)$$

Also, since

$$i_p = S\frac{1}{m}\left(\frac{dm}{dp}\cdot\frac{dx}{dx}\right)^2 = S\frac{1}{m}\left(\frac{dm}{dx}\right)^2\left(\frac{dx}{dp}\right)^2 = i_x\left(\frac{dx}{dp}\right)^2$$

and

$$i_p = i_x\left[\frac{d[p(1-p)]}{dp}\right]^2 = i_x[1 - 4p(1-p)] = i_x(1 - 4x),$$

the standard error of p then becomes

$$S.E._p = \sqrt{\frac{1}{I_x(1-4x)}}. \qquad (F)$$

When x is small, the standard error of p is not greatly different from the standard error of x, but as x approaches .25 the $S.E._p$ approaches infinity.

THE DETERMNIATION OF LINKAGE INTENSITIES

HANSON and KRAMER (1949) have presented the genetic analysis of two translocations in barley from F_2 data. In one of these interchanges (Accession 301), the chromosome carrying linkage group IV was shown to be involved. The other chromosome of the interchange complex could not be established with any degree of certainty; however, the VI linkage group was thought to be involved. The factor pairs hooded vs. awned (*K,k*) and normal vs. xantha seedlings (*Xc,xc*) in linkage groups IV and VI, respectively, (ROBERTSON WIEBE, and SHANDS 1947) were used to study linkage relationships.

For additional data in the F_3, dominant F_2 plants were randomly selected in the normal and semisterile classes, and F_3 progeny rows were grown from each selected plant. Classification of these rows established the genotypes of the F_2 plants. The F_2 data from HANSON and KRAMER (1949) and the additional F_3

TABLE 5

Linkage data in the F_2 and F_3 generations involving the interchange point in accession 301 and the factor pairs K,k and Xc,xc.

	INTERCHANGE 301 VS. *K,k*			INTERCHANGE 301 VS. *Xc,xc*		
F_2		*K−*	*kk*		*Xc−*	*xcxc*
	SS	a=207	c=23	SS	a=105	lethal
	N	b=140	d=87	N	b=89	lethal
F_3		*KK*	*Kk*		*XcXc*	*Xcxc*
	SS	e=13	f=138	SS	e= 4	f=101
	N	g=57	h= 36	N	g=65	h= 24

data are presented in table 5. The letters given in this table correspond to the appropriate observed classes in the formulas of tables 3 and 4.

The complete analysis for linkage between the interchange point and *k* is illustrated in table 6. The first step in the analysis of such data would be to determine whether the interchange break and the factor pair were independently inherited. When the observed values for a, b, c, etc., in table 5 are substituted into the appropriate formulas for the score in column 2 of table 4, the scores taken at x=.25 (table 6, column 2) are obtained. The I_x values appearing in column 3 of table 6 are obtained by multiplying the fractions for the amount of information i_x in column 3 of table 4 by the number classified for each source of data. These values are the total amount of information contributed by each class when x is taken at .25 for independence. The χ^2 values for independence (Expression B) correspond to the linkage component in a

TABLE 6

The calculation of linkage between the interchange point and the K,k locus.

SOURCE OF DATA	x=.25			x=.11		
	SCORE (c_x)	I_x	$\chi^2=\frac{c_x^2}{I_x}$	SCORE	I_x	χ^2
F_2 (Four classes)	−345.3	2437	48.93	−17.1	3294	.09
F_3 (SSA)	−298.7	2148	41.54	−66.0	2222	1.96
F_4 (NA)	−208.0	1323	32.70	+28.6	2913	.28
Sum χ^2			123.17			2.33
Total	−852.0	5908	122.87	−54.5	8429	.35
Homogeneity χ^2			.30			1.98
Estimate of x			.11			.104
Recombination value p						.12±.014

partitioned χ^2 for goodness of fit. Since the three values are very highly significant, the hypothesis of independence must be abandoned. In the absence of significant χ^2 values, the analysis is complete at this point. Since linkage exists in these data, the next step is to test for population homogeneity. From relationship C, the χ^2 for population homogeneity with 2 degrees of freedom becomes 123.17−122.87=.30, where the value 122.87 is obtained by computing a χ^2 for the totals of the c_x and I_x columns. With non-significance for this test, the data from the three sources are considered homogeneous and are combined. A provisional estimate of x is obtained by applying formula D. Thus .25−852.0/5908=.11. The value .11 may now be substituted for x in the formulas in columns 2 and 3 of table 3 and the procedure repeated with this revised x value. The second estimate for x yields .104 and a third estimate, which may be calculated as a check if desired, shows no significant improvement. Applying formula E,

$$p = \frac{1 - \sqrt{1 - 4(.104)}}{2} = .12.$$

The standard error of p may be approximated from formula F,

$$S.E._p{}^* = \sqrt{\frac{1}{8429(1 - 4\times.104)}} = .014.$$

The value of $p = .12 \pm .014$ is the best estimate of the recombination value which can be obtained from the combined data.

The analysis of the data involving the interchange break and the lethal factor *xc* is made in an identical manner and is presented in table 7. Based on the highly significant χ^2 tests for independence in the F_3 data, the hypothesis of independence between the xantha locus and the interchange point must be

TABLE 7

The calculation of linkage between the interchange point and the Xc,xc locus.

SOURCE OF DATA	x = .25			x = .07		
	SCORE (c_x)	I_x	$\chi^2 = \frac{c_x^2}{I_x}$	SCORE	I_x	χ^2
F_2 (Two classes)	− 21.3	345	1.32	+43.2	366	5.10
F_3 (SSA)	−248.0	1493	41.19	−64.9	2017	2.09
F_3 (NA)	−282.7	1266	63.13	+35.4	4550	.28
Sum χ^2			105.64			7.47
Total	−552.0	3104	98.16	+13.7	6933	.27
Homogeneity χ^2			7.48			7.20
Estimate of x			.07			.072
Recombination value p						.08 ± .014

abandoned, identifying the VI linkage group with the interchange complex. Evidence of heterogeneity between the different sources of data is apparent from the χ^2 value of 7.48 for homogeneity with 2 degrees of freedom which is significant at the 5 percent level. An inspection of the individual χ^2 values show that the F_2 data are out of line. This heterogeneity could be due to misclassification of semisterility on some of the F_2 plants since the two sources of F_3 data can be shown to be homogeneous.

It is of special interest to note that the information furnished by the two classes in the F_2 is very small compared to the amount of information furnished by the F_3 progeny lines. Because of this, the F_2 data will have little effect on the final recombination value and are included for illustration. The recombi-

* For illustration the value for I_x of 8429 is taken from table 5, where x = .11. A more accurate estimate would be made with I_x taken for x = .104, but this would not change the value of the $S.E._p$ appreciably.

nation value in this case is calculated to be $.08 \pm .014$. Thus, the use of seedling lethals in studies of this type is not precluded if the tests are carried into the F_3.

DISCUSSION

The study of linkage between a translocation involving two non-homologous chromosomes and the locus of a factor pair by the use of segregating data has been outlined. It has been demonstrated that the use of F_2 and F_3 data to obtain the linkage values between the break and a gene is feasible. Through the use of the x transformation and the method of scoring, the computations are reasonably simple. A method for the determination of linkage intensities which employs the F_3 as well as the F_2 is valuable because of the additional information available; the use of seedling lethal characters in studies of this kind is not precluded because of the lack of information supplied by the F_2. The method as used here is, of course, equally applicable when only a single source of data is available. If the F_2 data are handled in this manner, F_3 data may be added without the necessity of recalculating the F_2.

One of the major difficulties in the use of the method is one which is intrinsic in the data. As a result of the failure to separate genetically recombination and non-recombination gametes, p can not be determined directly by the applica-

TABLE 8

F_2 and backcross populations which on the average will give significant deviations from independence at the 5 percent level at given recombination percentages.

RECOMBINATION	SIZE OF POPULATION		RECOMBINATION	SIZE OF POPULATION	
PERCENTAGE	BACKCROSS	F_2	PERCENTAGE	BACKCROSS	F_2
0	4	12	25	15	184
5	5	18	30	24	450
10	6	28	35	43	1,420
15	8	48	40	96	7,200
20	11	89	45	384	115,200

tion of the maximum likelihood formulas. Hence, recombination values can be inferred only indirectly, the one chosen being that which is less than .5. That such an inference is correct, however, is easily demonstrated empirically for a single source of data by calculating expected phenotypic proportions using the inferred recombination value.

A second major difficulty is the loss of information suffered because of the limitations in classification. In table 8 the population sizes necessary on the average to detect a given recombination value at the 5 percent level using backcross and 4-class F_2 data are compared, ignoring the correction for continuity. In the range of 0 to 30 percent recombination the backcross furnishes from 3 to almost 20 times the information provided by the F_2. Above 30 percent recombination, the size of populations necessary to detect linkage with F_2 data rapidly becomes prohibitive. The use of F_3 data partially offsets this difficulty. At about 30 percent recombination the F_3 classes furnish 2 to 3 times the information obtainable from the F_2. The difficulty is also partially elimi-

nated in species which have primarily alternate disjunction of the ring of 4 at metaphase I of meiosis, since in these species, as HANSON and KRAMER (1949) have pointed out, recombination should be reduced in the arms of the synaptic complex carrying the centromere, the reduction depending on the length of interstitial region involved.

Where the use of backcrosses is feasible, such data are to be preferred to F_2 data; hence, situations in which it is desired to combine the two types of data probably will be rare. Since the backcross technique permits the identification of recombination gametes as in contradistinction to the use of F_2 and F_3 material, data from the two sources are not amenable to combining by the scoring method used here. If necessary, however, they can be combined by weighting the appropriate amounts of information furnished by each source.

SUMMARY

In determining linkage values from F_2 and F_3 data involving the point of interchange between non-homologous chromosomes and a factor pair, recombination and non-recombination gametes can not be identified by phenotypic classification, and coupling and repulsion phases can not be detected. Therefore, it is essential that linkage be studied as a function of both recombination and non-recombination gametes.

Using the quantity $x=p(1-p)$, formulas were derived which permit the necessary tests for independence and homogeneity and which facilitate the combining of data and the computing of linkage from F_2 and F_3 sources of genetic material. The method was illustrated in detail using a translocation in barley.

When some of the F_2 classes are eliminated because of seedling lethality, very little information is obtained in the F_2. This difficulty can be completely obviated when the studies are carried through the F_3 generation, permitting the use of seedling lethals as marker genes. It was possible to show that the chromosome carrying linkage group VI was involved in the interchange by using the seedling lethal conditioned by *Xc,xc* as a genetic marker.

Previous data which had established linkage group IV with the interchange complex were substantiated by F_3 data.

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SELF-INCOMPATIBILITY IN CREPIS FOETIDA (L.) SUBSP. RHOEADIFOLIA (BIEB.) SCHINZ ET KELLER

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SELF-INCOMPATIBILITY, or self-sterility caused by this condition, may be defined as the physiological inability of an organism possessing both functional male and female gametes to be self-fertilized. It refers only to instances in which the plant's own pollen and pistil come in contact with each other in the normal course of events, and excludes those cases in which self-fertilization is prevented by some natural device.

In the Compositae, a member of which is to be dealt with in this paper, very little work on self-sterility has been done. Only the work by STOUT on *Cichorium intybus* (1916, 1917, 1918), the observation by BABCOCK and HALL (1924) of intrasterile classes in *Hemizonia congesta*, a preliminary study of self-sterility in *Crepis foetida* by BABCOCK and CAVE (1938), and a brief study on *Cosmos bipinnatus* by LITTLE, KANTOR and ROBINSON (1940) were available when the work reported here was undertaken. In view of EAST's opinion (1940) that it is not unlikely that fully one-quarter of the approximately 14,000 species of the Compositae exhibit self-sterility to some degree, such paucity of data is surprising. The preliminary investigations of BABCOCK and CAVE had indicated that in *C. foetida* the situation in regard to self-sterility probably did not correspond to any of the previously known schemes. The present research was brought to a close in 1943, but the publication of a report has been unfortunately delayed. Meanwhile, GERSTEL (1950) has reported a similar genetic basis for self-incompatibility in guayule (*Parthenium argentatum*).

MATERIALS AND METHODS

The strain of *C. foetida rhoeadifolia* used in this study was grown from seed collected by DR. EDGAR ANDERSON in Yugoslavia in 1934. It was selected for this study from a number of other strains of this species because of its consistent and almost complete self-sterility, its short growing season and its abundance of large flowers.

Because of the nature of the flower and the large number of crosses involved, emasculation was impracticable. Preliminary study indicated that selfed heads very rarely set any seed and never over five percent of their potential capacity. Hence cross-pollination was carried on by bagging the heads prior to opening and massaging them together gently once each day from the time the outer circle of florets opened until the inner circle had opened. Thus, in the event of unfavorable weather a floret had more than one exposure to pollen. Flowers were kept bagged until the corollas had fallen. The possibility that compatible pollen on the stigmas might stimulate the plant's own pollen to

function is pretty well eliminated both by the experience of other workers on self-incompatibility and by a test carried on with Crepis. Since it was known that incompatible pollen fails to germinate, small amounts of compatible pollen were placed on stigmas and counted after application, with and without large quantities of the plant's own pollen. Later counts of the numbers of germinated pollen grains showed that addition of large amounts of the plant's own pollen to the small number of compatible grains did not increase the amount of germinated pollen.

Since Crepis pollen does not germinate on an incompatible stigma, it is possible to determine cross-relations between plants by an examination of stigmas a short time after they have been cross-pollinated. The florets being arranged concentrically, a head was selected whose outer circle had not yet begun to open and certain florets were removed, leaving three or four well-separated groups of three florets each. The style branches were then tapped gently to cause them to open out and expose the receptive part of the stigma. Pollen of a different male was placed on the stigmas of each group of florets, a pin being stuck into the head as a marker and the forceps being dipped in seventy percent alcohol between each pollen change.

Since Crepis pollen germinates rapidly, the stigmas were removed in about fifteen minutes, dipped several times in alcohol and then mounted in cotton blue lactophenol. If the cross was compatible a number of pollen tubes would have penetrated the stigma, firmly anchoring the pollen grains to the stigmatic surface. Both short sections of the tubes and many pollen grains would be visible. If, however, the cross was incompatible the alcohol wash would remove all pollen from the stigma because there would be no pollen tubes to hold them on. Occasionally a grain or two would be wedged in between some papillate cells and in a few instances a very small number of germinated grains were present, indicating slow or weak pollen tube growth.

With those plants whose cross relations were determined by this cytological method all determinations were duplicated and often triplicated. The method was found to be quite reliable. The cross relations of 43 of the 122 plants tested were determined by the cytological method but, of each family so tested, representative plants of each incompatibility group were thoroughly checked by actually performing the necessary crosses.

It was desired to retain at least one member of each homogeneous incompatibility group for use as a testing plant or "tester" in order to determine the behavior of untested plants. Vegetative propagation was accomplished by excising basal shoots, dipping them in hormodin and placing them in a propagating bed maintained at 25.6°C. Good rooting was obtained within two or three weeks.

PROCEDURE

From the seeds of *C. foetida rhoeadifolia* which were sown in the spring of 1939 only two plants reached maturity. These were self-incompatible but cross-compatible. Twenty plants were grown from this cross in 1940; all these plants were selfed and all possible crosses between sister plants were made and

TABLE 1

Cross Relations of H837

		MALE																				
		I					II				III					IV			UNCLASSIFIED		
	PLANT NOS.	1	5	8	12	15	2	3	16	20	6	7	10	13	19	11*	17	18	4	9	14
Female	1	S	S	S	S	S	S	S	S	S	F	F	F	F	F	S	S	S	S	F	S
		S	S	S	S	S	S	S	S	S	F	F	F	F	F	S	S	S	S	F	S
	5	S	S	S	S	S	S	S	S	S	F	F	F	F	F	S	S	S	f	F	S
		S	S	S	S	S	S	S	S	S	F	F	F	F	F	S	S	S	f	F	S
I	8	S	S	S	S	S	S	S	S	S	F	F	F	F	F	S	S	S	f	F	S
		S	S	S	S	S	S	f	S	S	F	F	F	F	F	S	S	S	S	F	S
	12	S	S	S	S	S	S	S	S	S	f	f	F	F	f	S	S	S	f	F	S
		S	S	S	S	S	S	S	S	S	f	f	F	f	f	S	S	S	S	f	S
	15	S	S	S	S	S	S	S	S	S	F	f	F	F	F	S	S	S	F	F	S
		S	S	S	S	S	S	S	S	S	F	f	F	F	F	S	S	S	S	F	—
	2	S	f	F	f	F	S	S	S	S	F	F	F	F	F	S	F	F	f	F	S
		F	S	S	f	f	S	S	S	S	F	F	F	F	F	S	f	F	f	F	S
	3	F	F	F	F	F	S	S	S	f	F	F	F	F	F	F	F	F	f	F	S
		F	f	f	F	F	S	S	S	S	f	F	F	f	F	S	f	F	S	F	S
II	16	F	F	f	F	F	S	f	S	S	F	F	F	F	F	f	F	F	S	F	—
		F	f	f	F	F	S	S	S	S	F	F	F	F	F	S	F	F	S	F	—
	20	F	F	F	F	F	S	S	S	S	F	F	F	F	F	S	F	F	S	f	—
		f	f	S	F	F	S	S	S	S	F	f	F	F	f	f	F	F	S	F	—
	6	F	F	S	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	—
		F	f	S	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	—
	7	F	f	F	F	F	F	F	F	F	S	—	S	S	S	S	S	S	S	S	S
		F	S	f	F	F	F	F	F	F	S	—	S	S	S	S	S	S	S	S	S
III	10	F	f	F	F	F	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S
		F	f	F	F	F	F	F	F	F	S	f	S	S	S	S	S	S	S	S	S
	13	F	F	f	F	F	F	F	F	F	S	S	S	—	S	S	S	f	S	S	—
		F	F	S	F	F	F	F	F	F	S	S	S	—	S	S	S	S	S	S	—
	19	F	f	f	F	F	F	F	F	F	S	S	S	S	S	S	S	f	S	f	—
		F	f	F	F	F	F	F	F	F	S	S	f	S	S	S	S	S	S	S	—
	11	S	S	S	S	S	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S
		S	S	S	S	S	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S
IV	17	S	S	S	S	S	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S
		S	S	S	S	S	F	F	F	F	S	S	S	S	S	S	S	S	S	S	—
	18	S	S	S	S	S	F	F	F	F	S	S	S	f	S	S	S	S	S	S	S
		S	S	S	S	S	F	F	F	F	S	S	S	S	S	S	S	S	S	S	S
Unclassified	4	F	F	S	F	F	f	f	S	S	f	S	S	S	S	S	S	S	—	S	S
		F	F	f	S	F	S	f	f	f	f	S	S	f	f	S	S	S	—	S	S
	9	F	f	F	F	F	S	S	S	S	S	S	S	S	S	S	S	—	S	S	S
		F	S	F	F	F	S	S	S	S	S	S	S	S	S	S	S	S	S	S	—
	14	F	F	F	F	S	f	F	—	—	—	S	S	—	—	S	—	S	—	—	S
		F	S	S	—	—	—	S	—	—	—	S	S	—	—	—	—	—	S	—	S

S: 0–10 seeds; f: 11–35 seeds; F: 36 or more seeds; —: no cross made.
The two letters in each section represent duplicate crosses.
* More than 97 per cent bad pollen.

duplicated. When it was found that these plants fell into homogeneous groups with regard to their cross relations, at least one family was grown from every cross between compatible groups. The individuals in each family were then crossed with testers representing each incompatibility group and classified according to their reactions.

Incompatibility was not always absolute so that a few seeds were occa-

sionally obtained from crosses that were obviously incompatible. All such seeds were grown and the plants were tested as to their cross relations.

RESULTS AND DISCUSSION

Table 1 shows the interrelations of the 20 original plants grown from the cross of the two self-incompatible, cross-compatible parents. Table 2 is a condensed record of the results of testing all of the individuals grown from crosses between members of the 20 offspring of the original two plants. In condensing this table some of the plants tested in Groups II, V, VI and VII were omitted. Their behavior was essentially the same as that of the others. The tested plants are grouped according to their compatibilities rather than their derivation. Table 2 will be found at the end of the paper.

Table 1 shows that the 20 plants from the original cross could be divided into four intra-sterile groups of homogeneous behavior. In the 15 families grown from crosses between members of this first family these same four intrasterile groups were found and, in addition, three other distinct and homogeneous incompatibility groups. This amounts to a total of seven homogeneous incompatibility groups arising from various combinations of the self-incompatibility genes present in the original two plants. The interrelations of these seven groups is as follows:

		MALE						
		I	II	III	IV	V	VI	VII
F	I	S	**S**	F	S	S	F	F
e	II	**F**	S	F	F	F	**F**	F
m	III	F	F	S	S	F	S	F
a	IV	S	F	S	S	S	S	F
l	V	S	F	F	S	S	F	F
e	VI	F	**S**	S	S	F	S	F
	VII	F	F	F	F	F	F	S

S: sterile; F: fertile. Reciprocal differences are shown in bold face.

The families grown during the course of the work, their parentage and the incompatibility groups found in their progenies are listed below:

FAMILY		PARENTS		GROUPS FOUND
H837	from	H821-1	×H821-2	I, II, III, IV
H893	from	H837-15 (I)	×H837-19 (III)	II, IV, V, VI
H880	from	H837-16 (II)	×H837-15 (I)	I, II, V
H892	from	H837-16 (II)	×H837-19 (III)	III, VI, VII
H891	from	H837-16 (II)	×H837-11 (IV)	I, V
H894	from	H837-2 (II)	×H837-18 (IV)	I
H895	from	H837-2 (II)	×H837-17 (IV)	V
H870	from	H837-19 (III)	×H837-15 (I)	II, IV, V, VI
H875	from	H837-19 (III)	×H837-16 (II)	II, III, VI, VII
H876	from	H837-19 (III)	×H837-17 (II)	II, III, VI, VII
H874	from	H837-11 (IV)	×H837-16 (II)	I, III, V, VI

The families grown from "incompatible" combinations were as follows:

FAMILY	PARENTS	GROUPS FOUND
H890	from H837-8 (I) ×H837-15 (I)	I
H889	from H837-8 (I) ×H837-3 (II)	II
H888	from H837-3 (II) ×H837-16 (II)	II, VII
H886	from H837-16 (II) ×H837-3 (II)	II, VII
H885	from H837-10 (III) ×H837-7 (III)	III, VII

Since the "oppositional factor" scheme of inheritance for self- and cross-incompatibilities is widely applicable in the plant kingdom it may be well to compare it with the behavior found in Crepis. In this scheme, as it operates in Nicotiana (EAST and MANGELSDORF 1926) and a great many other plants, there is a single series of alleles conditioning self- and cross-incompatibilities. The presence of any one of these alleles in the style of a plant inhibits the functioning of all pollen possessing the same allele. This causes all plants to be self-incompatible and gives reciprocal differences only when homozygotes are obtained by some device to break down the incompatibility barriers such as bud-pollination. If two plants are reciprocally compatible only two situations are possible among their progeny: either four inter-fertile, intra-sterile groups are obtained from the cross, all four of which are compatible with both parents; or (if the two parents have one incompatibility allele in common) only two inter-fertile, intra-sterile groups will be obtained, one of which will be fertile with both parents, the other fertile with only the female. All pollen behavior is gametophytic in that its behavior is determined by the incompatibility allele it is carrying.

Comparing the typical reaction between offspring and parents in Nicotiana with that found in two typical Crepis families:

Nicotiana—four intra-sterile, inter-fertile groups, all compatible with both male and female parents

Crepis —four intra-sterile groups, but compatibility with parents is as follows:

H870	H875
Group VI—incompatible with female compatible with male	Group III —incompatible with female compatible with male
Group V —compatible with female incompatible with male	Group II —compatible with female incompatible with male
Group IV—incompatible with female incompatible with male	Group VII—compatible with female compatible with male
Group II —compatible with female reciprocally different with male	Group VI —incompatible with female reciprocally different with male

The difference in the behavior of these two progenies from each other, as well as from Nicotiana, stresses the fact that in Crepis all families do not react

in the same way toward their parents. It is also apparent from the cross relations between the seven groups that, unlike Nicotiana where any group is compatible with all other groups and incompatible only within the group, in Crepis a group may be incompatible with one or several other groups besides its own.

The small number of groups obtained, even though the attempt was made to combine the incompatibility genes of the two original parents in as many ways as possible, suggests that incompatibility in Crepis is conditioned by a single series of alleles. Since members of different groups can be cross-incompatible, it seems probable that possessing only one allele in common is sufficient to cause incompatibility. But they behave differently because of the allele in which they differ, even to the extent of overcoming the incompatibility that might have been caused by the allele they have in common. Furthermore, if all of the pollen of a plant is inhibited on the stigmas of another plant which possesses only one allele in common, then that half of the pollen not carrying said allele must be inhibited. This is sporophytic determination; that is, the pollen behaves according to the genotype of the plant from which it came rather than according to its own genotype.

Therefore, the theoretical cause of reciprocal differences in Nicotiana and other plants, namely gametophytic determination of pollen behavior, will not explain reciprocal differences in Crepis. Another reason for its rejection is the doubt as to whether homozygotes are involved in the reciprocal differences in Crepis. The group which functions with another group as a female but not as a male should, according to the oppositional theory, be homozygous. However, group II, which might be considered as homozygous, is present in family H837 in the frequency of four out of a total of twenty plants, or twenty percent. It is also present in other families at a high frequency. Homozygosity, however, represents a breakdown or failure in the incompatibility mechanism and is expected to occur quite rarely except where special techniques are used to eliminate the barrier such as bud-pollination. Furthermore, if group II plants are homozygotes only group II plants would appear in their offspring, assuming that it was possible to get any offspring from crossing such plants. Progenies H886 and H888, however, originated in this way and each possesses group VII as well as group II plants. It appears, therefore, that group II is not homozygous and that homozygotes are probably not essential for the occurrence of reciprocal differences in Crepis.

It has been assumed that two incompatibility groups would be cross-incompatible if they possessed one allele in common. If the situation were so simple, then any group would be incompatible with both parents and with all but one of its three sister groups. Such regularity as this, or as that which occurs in Nicotiana, is not found in Crepis. Each group is not incompatible with the same number of other groups. Each homogeneous group or genotype appears to have its own particular strength or potency, which, since it is the same irrespective of the source of the particular plant, appears to reflect the different strengths of the individual alleles making up that genotype.

An explanation of the incompatibility behavior of the Crepis family de-

scribed above must include the following provisions: (1) The mechanism by which two plants of different genotypes can be cross-incompatible; (2) the means by which reciprocal differences occur; (3) the cause of the different strengths or potencies of the different genotypes. To meet these requirements the following hypothesis is proposed.

HYPOTHESIS

1. The reaction of the pollen is determined by the genotype of the plant from which it came rather than by its own genotype.

2. Four alleles of a single allelic series are responsible for the self- and cross-incompatibilities which have been found. They will be designated S_1, S_2, S_3 and S_4.

3. S_1 is recessive to the other three alleles. It is able to inhibit fertilization only when it is homozygous in both the male and the female involved. Therefore, any cross between two plants carrying it, if compatible, will produce some homozygotes among the offspring.

4. S_2 is dominant over S_1 but recessive to S_3 and S_4. Therefore, when present in a plant in combination with either of the latter two alleles the plant's reaction is determined by the dominant allele present. When it is present in combination with S_1 the plant's reaction is determined entirely by the S_2 allele.

The dominance relations or difference in potencies of the several alleles accounts for the reciprocal differences. For example, if two plants are both heterozygous for S_2 but in one it is associated with an allele to which it is recessive and in the other it is associated with S_1 the following result will be obtained: S_1S_2 ♀ × S_2S_3 ♂—compatible; S_2S_3 ♀ × S_1S_2 ♂—incompatible. This is because S_3 dominates in determining the pollen reaction in the first instance but S_2 dominates in the reciprocal cross.

5. S_3 and S_4 are "strong" alleles, both dominant over S_1 and S_2 but neither dominant over the other. Hence the presence of either or both in a plant causes its pollen to fail on plants containing like alleles.

GENOTYPES

In terms of the above hypothesis the cross relations between the seven groups and their hypothetical genotypes appear as follows:

		MALE						
		I (S_2S_4)	II (S_1S_2)	III (S_1S_3)	IV (S_2S_4)	V (S_1S_4)	VI (S_2S_3)	VII (S_1S_1)
	I(S_2S_4)	S	**S**	F	S	S	F	F
F	II(S_1S_2)	**F**	S	F	F	F	**F**	F
e	III(S_1S_3)	F	F	S	S	F	S	F
m	IV(S_2S_4)	S	F	S	S	S	S	F
a	V(S_1S_4)	S	F	F	S	S	F	F
l	VI(S_2S_3)	F	**S**	S	S	F	S	F
e	VII(S_1S_1)	F	F	F	F	F	F	S

Reciprocal differences are shown in bold face.

For further clarification of the segregation which occurred in the tested families, the following arrangement shows the probable genotypes of parent and offspring groups together with the number of plants of each genotype found in the progeny:

	P			F$_1$								
H837	V	×	VI	4	II	5	III	5	I	3	IV	
	(S_1S_4)		(S_2S_3)		(S_1S_2)		(S_1S_3)		(S_2S_4)		(S_3S_4)	
H893	I	×	III	2	II	3	VI	4	V	2	IV	
	(S_2S_4)		(S_1S_3)		(S_1S_2)		(S_2S_3)		(S_1S_4)		(S_3S_4)	
H880	II	×	I	4	II	2	V	?		2	I	
	(S_1S_2)		(S_2S_4)		(S_1S_2)		(S_1S_4)		(S_2S_2)		(S_2S_4)	
H892	II	×	III	2	VII	1	III	0	II	5	VI	
	(S_1S_2)		(S_1S_3)		(S_1S_1)		(S_1S_3)		(S_1S_2)		(S_2S_3)	
H891	II	×	IV	0	III	1	V	0	VI	4	I	
H894	(S_1S_2)		(S_3S_4)		(S_1S_3)		(S_1S_4)		(S_2S_3)		(S_2S_4)	
H895												
H870	III	×	I	9	II	5	V	4	VI	10	IV	
	(S_1S_3)		(S_2S_4)		(S_1S_2)		(S_1S_4)		(S_2S_3)		(S_3S_4)	
H875	III	×	II	10	VII	7	II	2	III	5	VI	
H876	(S_1S_3)		(S_1S_2)		(S_1S_1)		(S_1S_2)		(S_1S_3)		(S_2S_3)	
H874	IV	×	II	2	III	2	VI	3	V	2	I	
	(S_3S_4)		(S_1S_2)		(S_1S_3)		(S_2S_3)		(S_1S_4)		(S_2S_4)	
Those from "incompatible" combinations were:												
H890	I	×	I	0		1	I	1	V ?			
	(S_2S_4)		(S_3S_4)		(S_2S_2)		(S_2S_4)		(S_4S_4)			
H889	I	×	II	1	II	0		0	V	0	I	
	(S_2S_4)		(S_1S_2)		(S_1S_2)		(S_2S_2)		(S_1S_4)		(S_2S_4)	
H886	II	×	II	3	VII	7	II	?				
H888	(S_1S_2)		(S_1S_2)		(S_1S_1)		(S_1S_2)		(S_2S_2)			
H885	III	×	III	2	VII	2	III	?				
	(S_1S_3)		(S_1S_3)		(S_1S_1)		(S_1S_3)		(S_2S_3)			

It should be noted that the progeny of H891 also included one group II and one group VII plant. Since the male parent (H837-11) of this family possessed over 97 percent bad pollen both plants may have arisen through self-fertilization.

It is apparent that in those instances when S_2S_2, S_3S_3, or S_4S_4 plants were obtained they could not be distinguished by their compatibilities from S_1S_2, S_1S_3 or S_1S_4, respectively.

The hypothesis proposed and elaborated above includes the three essential provisions called for (p. 576) as shown below:

1. Two plants belonging to different groups can be cross-incompatible because they have an incompatibility allele in common. Since determination of pollen behavior is sporophytic, all of the pollen of a plant is inhibited on another's style if there is one allele in common.

2. Reciprocal differences are caused not by gametophytic determination and homozygosis, but by unequal potency of the alleles or dominance in the

sporophytic determination of pollen behavior. The dominance relations do not appear to hold in the stylar tissue or stigma, since the presence in such tissue of S_2 together with S_4 is capable of inhibiting the germination of S_2 pollen from an S_1S_2 plant (see H889, p. 577).

3. Intra-incompatible groups are incompatible with various other groups because of the difference in potencies of the alleles present in the incompatibility genotype. Six heterozygotes are possible when dealing with one series of four alleles and when all plants originate from the same two original parents directly or indirectly.

Two of these heterozygotes, groups III and V, which possess a strong allele plus the weak one, will each be incompatible with the other two groups possessing the same strong allele.

The two groups, I and VI, which possess a strong allele plus S_2, will likewise be incompatible with the two groups possessing the same strong allele but reciprocally different with group II.

Group IV, which has both strong alleles, will be incompatible with all four groups which have either one.

Group II, the remaining heterozygote, will be fertile as a female with all other heterozygous groups because only its own pollen is dominated by the S_2 allele. For the same reason it will be incompatible as a male on all other groups possessing the S_2 allele.

Group VII will be fertile with all other groups because it is homogyzous for S_1, the weakest of the four alleles, a double dose being needed in both male and female to cause inhibition.

Group VII is the only homozygote found among the plants tested, but homozygous S_2 plants should be possible, since plants containing S_2 and differing in their strong allele are compatible. Homozygous S_2 plants are not distinguishable from group II in their behavior and so would have to be identified by their offspring.

SUPPORTING EVIDENCE

The assumptions made to explain the data are in line with ideas advanced from time to time by other workers in this field. RILEY (1932, 1934, 1936) working on *Capsella grandiflora*, found it necessary to assume sporophytic determination of pollen behavior in order to explain the incompatibility phenomena in that species. LEWIS (1947) has shown that some incompatibility alleles in *Oenothera organensis* exert dominance over others. Self- and cross-incompabitilities in this species are conditioned by a single set of alleles according to the oppositional-factor scheme. Since determination of pollen behavior is gametophytic, dominance is possible only after tetraploidy has been induced and two alleles are thus present in the pollen grains. LEWIS showed that S_4S_6 pollen functioned on styles containing S_4, but not on styles containing S_6. Since as much seed was obtained when this pollen was used on styles containing S_4 as when used on styles containing neither allele, dominance was believed to be complete. LEWIS also found other instances in which one allele is domi-

nant over another in the pollen grain. He does not, however, find dominance being exerted in the style and points out that this would not be expected, since dominance in the style would cause the whole incompatibility mechanism to break down. If dominance occurs in a plant whose pollen behavior is gametophytically determined, even though it can manifest itself only after the chromosome number is doubled, it seems legitimate to assume that it can also occur in Crepis, whose pollen behavior is sporophytically determined. Likewise, it is in line with LEWIS' data on Oenothera to assume that dominance occurs in the pollen but not in the style.

The concept that some *S* alleles are weaker in potency than others is also in agreement with observations on other plants. For example, EAST (1929), in commenting on STOUT's failure to get regular behavior in *Cichorium intybus*, suggested that STOUT was working with alleles which were on the borderline between self-compatibility and self-incompatibility and could often be tipped in one direction or the other by environmental influences within or without the plant. Probably S_1 and S_2 of the present study come more or less within the category of borderline factors. In an evolutionary sense they might be thought of as intermediate steps between the primitive condition of self-compatibility and the advanced one of self-incompatibility.

ANALYSIS OF CERTAIN FAILURES OF THE DATA TO CONFORM TO THE HYPOTHESIS

Table 1, representing the interrelationships of 20 sister plants, conforms fairly well to the expected behavior except for three plants. Plants 4 and 14 were apparently somewhat male sterile (a phenomenon frequently encountered in the subsequent generation) and plant 9 deviated from group III behavior by being incompatible with group II. It is possible that all three of these plants could be placed in group III but, since there is some doubt about them, they are classified separately.

Table 2 is a compilation on the basis of behavior rather than family lines. In assembling the data on which this table is based certain deviations from expectation were noted and some accounting for these is in order. In the results of 2037 pollinations it was found that 356 deviate from the expected behavior. These deviations may be classified as follows:

1. FS—expected to be compatible but in one or more tests incompatible.
2. Ff—expected to be compatible but in one or more tests only feebly so.
3. SF—expected to be incompatible but in one or more tests compatible.
4. Sf—expected to be incompatible but in one or more tests feebly compatible.

It is apparent that a certain number of the first two types of deviation from expected may be due to fluctuations in the environment. Although the study of self- and cross-incompatibilities should properly be carried on under reasonably constant environmental conditions this ideal is rarely attainable in practice. In the present study it was customary to carry on pollinations nearly every day and at any season of the year. Although all of the work was

done in the greenhouse it was not possible to control light and humidity at all and the temperature varied within a fairly wide range. Another factor of importance was the physiological condition of the plant. On many occasions one plant needed for a cross was just beginning to bloom while the other was approaching senility. It is therefore to be expected that a combination which should have been fully compatible might occasionally fail completely or show only weak fertility due to the external or internal environment.

As noted in table 2, there were several plants which failed to function as males and whose pollen was later found to be bad. H893-5 and -9 apparently belong in this group, although their pollen was not examined. Plant H837-11, however, had over 97 percent bad pollen and yet it behaved erratically in the 36 crosses attempted with plants of groups II and VII with which it would be expected to be fertile. In two of these crosses enough seeds were produced to call for classification as F; but most of the remainder yielded from 0 to nine seeds, there being only eight that produced from 12 to 34 seeds and so would be classified as f. This plant was completely sterile when used as a male with plants of all the other groups. Since it shows partial fertility with groups II and VII it was placed in group IV. The two instances of high fertility in spite of the low percentage of good pollen grains remain unexplained.

FS and Ff. Although 260 of the 356 deviations from expected were FS or Ff, only 210 of these represented different plant combinations. 102 of these behaved according to expected when duplicated or, in a few cases, reduplicated. These can therefore largely be attributed to unfavorable internal or external environmental conditions at the time of the first pollination. Of the remaining 108 cases 81 were not duplicated. Many of these likewise were caused, no doubt, by environmental conditions.

The 27 cases of FS and Ff which persisted after duplications are probably genetic rather than environmental. Of the 54 parents involved, group VII contributed 12, group II contributed 11 and group V, 11. The other groups contributed five parents each. Not only were these deviations from expected more closely associated with certain groups, but also with certain individual plants. Plant H870-21 was a parent in ten of these combinations, H875-21 in five, and H876-17 in three others. In other words, a genetic difference in three plants could account for 18 of the 27 deviations. Of the 26 individual plants involved in these 27 deviations, 13 were involved more than once.

SF and Sf. SF reactions are the most serious deviations from expectation, since it is hardly likely that environment would alter an incompatible reaction to that extent. In the 27 cases of SF only 17 different crosses are involved. Ten of these are between group VII plants, and seven involve combinations between groups III, IV, and VI. No reason is apparent why groups I, II, and V are not represented in this type of deviation from expectation. However, the aberrant behavior of the ten combinations between group VII plants is in keeping with our concept of the S_1 allele for which group VII is homozygous. It is considered to be a weak allele which acts as a barrier to fertility only when homozygous in both male and female. If it is a borderline allele, then it is probable that minor genes in the plant's genotype might be able to alter the

incompatibility response. If two plants alike in being homozygous for S_1 were sufficiently unlike in other components of their genotypes, this might move the threshold below the level needed for cross-incompatibility.

Of the 69 cases of Sf, involving 58 different plant combinations, 32 reacted according to theory when duplicated; 15 were not duplicated. Eleven persisted in the f reaction when the cross was repeated.

The deviations of the FS and Ff type are believed to have been caused in large part by environmental fluctuations, internal as well as external to the plants. The SF responses and part ot the Sf are probably genetic in nature and exhibit the influence of minor genes which alter the incompatibility reaction, particularly when the *S* allele in the principal allelic series happens to be one of low potency.

THE CREPIS-PARTHENIUM SYSTEM OF SELF-STERILITY COMPARED WITH OTHER KNOWN SYSTEMS

Is the genetical system controlling self-sterility which has been discovered in Crepis and Parthenium unique? LEWIS (1944) made a helpful review of the known systems for self-sterility in plants. His treatment may be summarized as follows:

I. Heteromorphic (heterostyled) plants: *Primula sinensis* and many other investigated species.

1. One or two genes with two alleles.
2. Co-ordinated gene action in pistil and pollen.
3. Diploid pollen control.
4. Dominance.

II. Homomorphic (non-heterostyled) plants: *Nicotiana Sanderae* and all other investigated species, except *Capsella grandiflora*, *Crepis foetida* and *Parthenium argentatum*.

1. One gene with multiple alleles.
2. Independent gene action in the pistil but not in the pollen.
3. Haploid pollen control.
4. No dominance.

LEWIS points out that *Capsella grandiflora*, a non-heterostyled species, possesses all the genetical characteristics of the heterostyled system. Thus, a striking exception to the general rule has already come to light (RILEY 1936). Now, from investigations on two species, representing widely different tribes of the Compositae, the evidence calls for the following system:

III. Crepis and Parthenium.

1. One gene with multiple alleles.
2. Independent gene action in the style.
3. Diploid pollen control.
4. Dominance in the anther.

The genetical system controlling self-sterility in Crepis and Parthenium, therefore, is unique in that it is a combination of features from the two sys-

tems of homostyled and heterostyled plants. It may be noted that LEWIS (1944) dismissed the likelihood of the existence of such a system as the one proposed by GERSTEL and the present authors as hardly to be imagined. The only serious difficulty involved, however, concerns the second and fourth features: independent gene action in the pistil and dominance in the anther. In discussing the physiological control of incompatibility in plants, LEWIS states (p. 582) that "plants without heterostyly and heterostyled plants differ in a way which reflects the genetical differences. . . . Only one mechanism has been found in plants without heterostyly, and this appears to be an immunity type of reaction between specific proteins of the pollen and style. This alone has the possibility of variation necessary to meet the requirements of a large multiple allele series and the specificity necessary for the independent gene action in the style."

Since the specificity of proteins is practically unlimited, is it not conceivable that in the Compositae, the most highly evolved division of the Angiosperms, protein relations have developed which provide just the types of specificity required for independent gene action in the style or stigma and dominance in the anther? It should be remembered that, under normal conditions, no Crepis pollen will germinate on an incompatible stigma regardless of which allele a particular pollen grain carries. This fact suggests the possibility that in the Compositae the physiological incompatibility mechanism involves differences in osmotic pressure as in *Linum grandiflorum* (LEWIS 1943).

In this connection a statement of GERSTEL (1950) is worthy of repetition: "The reaction is one between cytoplasm produced in the male organ and a pistil. One cannot assume, however, that the same incompatibility substance, or substances, are diffused through the protoplasm of the entire plant, since an incompatibility *reaction* could then not take place; there must be two different substances which are able to react with each other, synthesized at two different places, the androecium and the gynoecium, by the same gene (or, as LEWIS [1949] recently postulated, by two self-reproducing parts between which no crossing over takes place)."

LEWIS (1949) also reports that radiation experiments show that the *S* gene behaves toward X-rays as though it were two independent units, one determining pollen reaction and one determining style reaction. And he alludes to other evidence from polyploid plants which indicates the dual nature of the *S* gene. This concept that the *S* gene is a "dual" entity may prove to be a fruitful idea in future research on self-sterility in the Compositae. But of equal interest is an answer to the question as to whether the genetical system controlling self-incompatibility in Crepis and Parthenium exists in many other genera, representing all the various tribes, of this great family.

SUMMARY

1. A cross between two self-incompatible plants of *Crepis foetida rhoeadifolia* yielded four intra-sterile groups in its progeny.

2. Some groups were inter-sterile and some were not. Reciprocal differences were found.

3. Progenies were grown from all possible combinations of the four original groups. They were tested by crossing with representatives of all available groups.

4. Seven incompatibility groups in all were found in the F_1 and F_2 from the two original plants.

5. These groups ranged in cross relations from one group which was compatible with all groups but its own, to one which was incompatible with all groups but two.

6. The self- and cross-incompatibilities in this strain of *C. foetida rhoeadifolia* can be explained genetically on the assumption that a single series of oppositional alleles is responsible. They may be referred to as S_1, S_2, S_3, and S_4.

7. It is postulated that:

a. Pollen behavior is sporophytically or diploid determined. That is, the behavior of a pollen grain is dependent upon the genotype of its parental sporophyte.

b. S_1 is recessive to all three of the other alleles. S_2 is dominant to S_1 but recessive to S_3 and S_4. S_3 and S_4 are both dominant to the weaker alleles but either is capable of complete expression in the presence of the other. Dominance is not expressed in the pistil.

c. Reciprocal differences are due to dominance, not, as in other known cases, except Parthenium, to homozygosity of one parent.

8. Other genes are believed to be able to exert some modifying effects, especially when associated with the S alleles of weaker potency.

9. The Crepis-Parthenium type of self-sterility is compared with other known types and is found to be unique.

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TABLE 2

Cross Relations of Derivatives of H837.

TABLE 2

Cross Relations of Derivatives of H837.

I	TESTERS																
	I	II			III	IV			V				VI			VII	
	H837	H837	H870	H870	H837	†H837	H870	H870	H874	H870	H870	H893	H870	H875	H876	H875	H875
	15	16	11	17	19	11	3	9	9	29	21	1	4	22	1	18	21
*H874-12	S	S	S	—	F	—	S	S	—	—	—	—	F	F	F	F	—
H874-24	S	S	—	—	F	—	S	S	—	—	—	—	F	—	—	—	F
*H874-7	SS	SS	SS‡	—	FF	—	—	SS	—	—	SS	—	FS	—	—	—	S‡F
H874-6	ff	ff	Sf	—	FF	—	fS	SS	—	—	SS	—	FF	—	—	F	FF
	SS	Ff	Sf	—	FF	—	SS	SS	—	—	—	—	FS	—	—	F	FF
*H880-4	SS	SS	—	—	FSF	—	S	SSS	—	—	S	—	F	FFS	—	FFS	—
*H880-5	S	S	S	—	F	—	S	S	—	—	—	—	F	F	F	F	—
H890-2	S	S	—	—	F	—	S	S	—	—	—	—	F	—	—	—	F
H890-5	SS	SS	S	—	FF	—	—	SS	—	—	S	S	F	SF	—	F	F
	-S	-F	F	—	-f	—	—	-S	—	—	S	-	-	Sf	—	F	-
*H891-6	S	S	—	—	F	—	—	S	—	—	—	S	F	—	—	F	—
H891-9	S	S	—	—	F	—	—	S	—	—	—	—	F	—	—	—	—
*H891-12	SSS	S	S	—	FSS	—	S	SS	S	—	S	S	FF	FF	—	F	F
H894-2	S	S	—	—	F	—	S	S	—	—	—	—	S	F	—	—	F
II																	
H870-11	FFFF	SSSS	—	—	FF	SS	F	FFF	—	F	S	—	FS	—	—	—	FF
	SSSS	fSS	—	—	FF	S‡F	—	F	—	F	F	—	—	—	—	—	—
H870-15	—	SS	—	S	FF	Sf	—	F	—	F	S	—	—	—	—	—	—
	—	fS	—	S	FF	-F	—	S	—	F	S	—	—	—	—	—	—
H876-21	F	S	S	—	F	—	F	F	—	—	—	—	F	F	F	F	—
H876-16	FF	SS	SS	—	SF	—	F	Ff	—	—	SS	—	fF	F	—	FF	FF
	ff	SS	Sf	—	ff§	—	F	FS	—	—	SS	—	S‡S	S	—	FF	FF
H880-2	F	S	-	—	F	—	F	F	F	—	—	—	F	F	-	F	-
	S	S	S	—	S	—	F	S	-	—	—	—	S	S	S	-	S
H880-24	F	S	S	—	F	—	F	F	—	—	—	—	F	F	F	F	-
	S	S	S	—	F	—	F	F	—	—	—	—	S	-	S	-	F
H880-26	FF	SS	S	—	FF	—	FF	FF	—	—	—	—	FF	F	F	F	-
	SS	Sf	S	—	FF	—	FF	FF	—	—	—	—	SS	S	S	-	F
H886-5	FF	SS	—	—	FF	—	F	FF	F	—	fS	—	FF	FS	—	F	-ff
	SS	SS	—	—	FF	—	F	FF	-	—	FF	—	SS	-S	—	-	FFf
H886-16	F	S	—	—	F	—	F	F	F	—	—	—	F	F	F	F	—
	S	S	—	—	F	—	-	F	F	—	—	—	S	-	-	S	—
H886-17	F	S	—	—	F	—	F	F	F	—	—	—	F	F	F	F	-

Upper letters refer to results obtained when tester plants were used as males; lower letters, when testers were used as females; two or more contiguous letters indicate duplicate or reduplicate tests.

S-0–10 seeds; f 11–34 seeds; F-35 or more seeds.

* Male sterile.

† H837-11 was largely male sterile but was sometimes erratic, in contrast with other male sterile plants.

‡ Results considered unreliable because the female parent died, or the branch containing the seed was broken or injured, shortly after pollination.

§ Flower very small which accounts for the small amount of seed, that is, the percent of florets which set seed was relatively high.

Note: In condensing the above table 15 tested plants were omitted from Group II, four from Group V, nine from Group VI, and seven from Group VII. Their behavior was essentially the same as that of the others.

TABLE 2—*Continued*

II	TESTERS																
	I	II			III	IV			V				VI			VII	
	H837 15	H837 16	H870 11	H870 17	H837 19	†H837 11	H870 3	H870 9	H874 9	H870 29	H870 21	H893 1	H870 4	H875 22	H876 1	H875 18	H875 21
	S	S	—	—	F	—	F	F	F	—	—	—	S	S	-	F	S
H888-4	F	S	—	—	F	—	F	F	—	—	—	—	F	S	—	F	-
	S	S	—	—	F	—	F	F	—	—	—	—	S	-	—	-	F
*H888-8	F	S	—	—	F	—	F	F	—	—	—	—	F	F	—	F	—
H889-1	FFS	SSS	—	—	FFf§	—	F	FF	f	—	Sff	—	F	FFf§	—	fS§	-f§
	SSS	SSS	—	—	FSF	—	F	FS	F	—	F-f	—	S	SSS	—	FF	FS
H891-8	F	S	—	—	F	—	F	F	—	—	—	—	—	—	—	—	—
*H893-7	F	S	—	—	F	—	F	F	F	—	—	—	F	F	—	F	—
H893-8	FFf	SSS	—	—	FFFF	—	F	FFS	F	—	SSS	—	FS	FF	—	FF	F
	SSS	SSS	—	—	FfSf	—	-	FFS	F	—	-FF	—	SS	-S	—	SF	S
III																	
H875-28	F	F	—	—	f	S	—	—	—	—	—	—	—	—	—	—	—
	F	F	—	—	S	S	—	—	—	—	—	—	—	—	—	—	—
H874-30	F	F	—	—	S	—	S	S	—	—	—	F	S	—	—	—	F
H874-44	FF	FF	F	—	SSS	—	S	SSS	—	—	SSF	f	S	SS	—	-	FF
	-F	-S	S	—	-SS	—	-	-SS	—	—	fFS	-	-	SS	—	F	-
*H875-8	FFFF	FfF	SF	—	SSS	—	—	SSS	—	—	SSS	—	—	S	—	F	—
H876-25	F	F	—	—	S	—	—	S	—	—	—	—	—	—	—	—	—
H885-12	FFF§	Ff	—	—	SSS	—	S	SSS	F	—	F	—	S	SS	—	FF	S
	FFf	Ff	—	—	fSS	—	-	SSS	F	—	F	—	S	-S	—	SF	S
H885-14	F	F	—	—	S	—	S	S	—	—	—	F	S	—	—	—	F
*H892-14	Fff	FfS	—	—	SS	—	S	SSS	—	—	SS	F	SS	—	—	F	FS
IV																	
H870-1	—	F	—	—	SS	fS	—	S	—	S	—	—	—	—	—	—	—
	—	F	—	—	Sf	SS	—	S	—	f	—	—	—	—	—	—	—
H870-6	—	F	—	—	SS	SS	—	S	—	S	—	—	—	—	—	—	—
	—	F	—	—	SS	SS	—	S	—	S	—	—	—	—	—	—	—
H870-7	—	F	—	—	SS	SS	—	S	—	S	—	—	—	—	—	—	—
	—	F	—	—	ff	SS	—	S	—	f	—	—	—	—	—	—	—
H870-8	—	F	—	—	SS	SS	—	S	—	S	—	—	—	—	—	—	—
	—	F	—	—	Ff	SS	—	S	—	S	—	—	—	—	—	—	—
H870-9	SfSf	F	FF	—	SS	SS	—	—	—	S	SS	—	SS	—	—	S	fS
	SSSS	F	—	—	f-	SS‡	—	—	—	S	S	—	—	—	—	—	—

TABLE 2—*Continued*

IV	TESTERS																
	I	II			III	IV			V				VI			VII	
	H837 15	H837 16	H870 11	H870 17	H837 19	†H837 11	H870 3	H870 9	H874 9	H870 29	H870 21	H893 1	H870 4	H875 22	H876 1	H875 18	H875 21
H870-10	—	F	—	—	SS	SS	—	S	—	S	—	—	—	—	—	—	—
	—	F	—	—	-f	SS	—	S	—	S	—	—	—	—	—	—	—
H870-19	—	F	—	F	ff	SS	—	S	—	S	—	—	—	—	—	—	—
	—	F	—	f	fS	SS	—	S	—	f	—	—	—	—	—	—	—
H870-23	—	F	—	f	ff	SS	—	S	—	S	S	—	—	—	—	—	—
	—	F	—	F	SS	SS	—	S	—	S	S	—	—	—	—	—	—
H870-27	—	F	—	—	Sf	SS	—	S	—	S	S	—	—	—	—	—	—
	—	F	—	—	fS	S‡S	—	S	—	S	S	—	—	—	—	—	—
H893-4	SSS	FFfS	—	—	SSS	—	S	SSS	S	—	SS	—	SS	SS	—	F	Ff
	SSS	FFSS	—	—	SSS	—	—	SSS	S	—	SS	—	SS	-S	—	S	ff
H893-2	S	F	—	—	S	—	S	S	S	—	—	—	f	f	F	F	—
	S	f	—	—	S	—	-	S	S	—	—	—	S	-	-	-	—
V																	
H870-3	SS	F	F	—	fF	SS	—	f	—	S	—	—	—	—	—	—	—
	SS	F	—	—	fF	SS	—	S	—	S	—	—	—	—	—	—	—
H870-21	S–S	FS	—	f	FF	SS	—	S	—	S	—	—	F	—	—	F	FF
	SSSS	FS	—	f	ff	SS	—	S	—	S	—	—	—	—	—	—	—
H874-9	SSS	FFF	FF	—	FSF	—	SfS	SSS	—	—	—	—	FFF	F	F	FF	–FF
	SSS	FFF	FF	—	FfF	—	SfS	SSS	—	—	—	—	FFF	F	F	–F	F–F
*H874-18	S	F	—	—	F	—	S	S	—	—	—	—	F	F	—	F	—
H874-38	S	F	—	—	F	—	S	S	—	—	—	—	F	—	—	—	S
H880-25	SS	F	F	—	FSF	—	—	SSS	—	—	SS	S	F	SF	—	-F	f
	SS	F	F	—	FSF	—	—	SSS	—	—	SS	S	F	fF	—	Ff	-
*H880-27	S	F	—	—	F	—	S	S	S	—	—	—	F	F	—	S	—
H890-6	SS	FF	—	—	FFF	—	—	SS	—	—	SS	—	F	FFF	—	S	FF
	-S	–F	—	—	–Sf	—	—	-S	—	—	SS	—	-	–FF	—	-	–S
H893-1	SSS	FfFS	F	—	FFff	—	SS	SSS	—	—	SS	—	FFF	F	F	FfFS	—
	SSS	FFFF	—	—	FSSf	—	S-	SSS	S	—	SS	—	FSF	F	—	FSS‡f	S
H893-5	S	F	F	—	F	—	S	S	—	—	—	—	F	F	F	F	—
	S	S	S	—	S	—	S	S	—	—	—	—	S	S	S	—	S
H893-9	S	F	F	—	F	—	S	S	S	—	—	—	F	F	F	F	—
	S	S	—	—	S	—	—	S	S	—	—	—	S	—	—	S	—
*H893-13	S	F	—	—	F	—	S	S	S	—	—	—	F	F	F	F	—
H895-5	SS	Ff	F	—	FSff	—	S	SSS	—	—	SSS	—	S	S	—	S	FS
	-S	–F	S	—	–SFS	—	—	-SS	—	—	SSS	—	—	f	—	f	–f

TABLE 2—*Continued*

VI	TESTERS																
	I	II			III	IV			V				VI			VII	
	H837 15	H837 16	H870 11	H870 17	H837 19	†H837 11	H870 3	H870 9	H874 9	H870 29	H870 21	H893 1	H870 4	H875 22	H876 1	H875 18	H875 21
H870-4	FFFF	fSSS	SS	—	SS	SS	—	SSS	—	F	F	—	—	S	—	—	SF
	FFFF	FFFF	—	—	SS	SS	—	S--	—	F	–	—	—	–	—	—	–
H875-22	FFF	SSS	S	—	SSSSf	S	S	S	F	—	—	—	SS	S	S	F	–
	SFF	FFF	F	—	SSSF	S	S	S	–	—	—	—	S	–	S	–	F
H875-25	fF	fS	—	—	SS	S	S	S	F	—	—	—	S	S	S	F	—
	F–	S–	—	—	S–	S	–	–	–	—	—	—	—	–	–	–	—
H874-5	F	S	S	—	–	—	–	SS	—	—	F	—	—	—	—	—	FF
	fS	F	F	—	S	—	S	SS	—	—	S‡	—	S	—	—	—	Sf
H876-1	FS	ff	f	—	fS	—	SS	ff	—	—	F	—	FS	—	—	FF	F
	FF	FF	F	—	S‡S	—	Sf	SS	—	—	–	—	SS	—	—	FS‡	F
H892-6	FFF	SSS	S	—	SSS	—	S	SSf	—	—	SF	—	SS	Sf	—	FF	–F
	FFF	FS–	F	—	SSS	—	S	SSS	—	—	FS	—	SS	-S	—	–F	FF
H892-7	F	S	—	—	S	—	S	S	—	—	—	f	S	S	—	F	—
	F	F	—	—	S	—	-	S	—	—	—	F	S	-	—	F	—
H892-11	F	S	—	—	S	—	S	S	-	—	—	F	S	S	—	F	—
	F	F	—	—	f	—	-	S	F	—	—	-	S	-	—	S	—
H892-13	F	S	—	—	S	—	S	S	F	—	—	F	S	S	—	F	-
	F	F	—	—	S	—	S	S	-	—	—	-	S	-	—	-	F
H892-15	F	S	—	—	S	—	S	S	—	—	—	F	S	S	—	F	-
	F	F	—	—	S	—	S	S	—	—	—	-	S	-	—	-	F
H893-12	FFF	SSSS	S	—	SfFs	—	SS	SfS	F	—	ff	—	Sf	SS	S	F	–FF
	FFF	FFFf	F	—	SSFS	—	S-	SSS	F	—	FF	—	SS	SS	-	-	FFF
VII																	
H875-18	SFS	FF	—	—	SF	—	—	F	—	—	F	—	—	—	—	—	S
	fF–	FF	—	—	FS	—	—	—	—	—	—	—	—	—	—	—	—
H875-21	SFS	FFF	FF	—	FF	S	—	SS	—	—	SF	—	SF	—	—	Sf	SS
	SFS	FFF	—	—	FF	F	—	--	—	—	—	—	—	—	—	—	—
H876-17	SS	FS	fF	—	FS	—	FF	FS	—	—	SS	—	SF	—	—	F	FS
	fS	Ff	Sf	—	FS	—	FF	FS	—	—	SF	—	SF	—	—	F	fS
H876-14	—	F	FS‡	—	FS	—	—	S‡F	—	—	S	—	FS‡	—	—	—	FS
	—	F	FS	—	F$S	—	—	S S	—	—	F	—	SS	—	—	—	Ff
*H885-2	F	F	—	—	F	—	—	F	—	—	—	F	F	—	—	—	S
*H885-10	FF	FF–	—	—	FFF	—	—	FSF	—	—	S	F	F	FF	—	—	SS
H886-1	FF	FF	—	—	FFF	—	F	FF	—	—	F	-	F	FF	—	F	F
	FF	FF	—	—	FFF	—	-	Ff	—	—	F	F	F	–F	—	F	F
*H891-4	F	F	—	—	F	—	—	F	—	—	—	—	F	F	—	F	—
*H892-10	FFF	FF	—	—	FSS	—	—	FSf	—	—	f	F	FS	S	—	FS	F
H886-15	F	F	—	—	F	—	F	F	—	—	—	-	F	F	—	F	—
	F	F	—	—	F	—	-	F	—	—	—	F	F	-	—	F	—
H888-9	F	F	—	—	F	—	—	F	—	—	—	—	F	—	—	—	F

CONTRIBUTIONS TO THE EVOLUTIONARY GENETICS OF THE LADY-BEETLE, HARMONIA. I. GEOGRAPHIC AND TEMPORAL VARIATIONS IN THE RELATIVE FREQUENCIES OF THE ELYTRAL PATTERN TYPES AND IN THE FREQUENCY OF ELYTRAL RIDGE*

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REVIEW OF GENETICS OF ELYTRAL PATTERN IN LADY-BEETLES

THE lady-beetles have been used by many workers as materials for genetic study, because of the high variability of their elytral and pronotal patterns. The previous papers on this subject were thoroughly reviewed by SHULL (1943). The differences in these markings in all cases so far worked out are due to multiple allelic genes. The expression of these genes is much dependent on external conditions to which the larva and pupa are subjected, high temperature and low humidity tending to decrease the extension of melanic pigment, especially tn the elytra, while low temperature and high humidity tend to increase it (JOHNSON 1910, ZIMMERMANN 1931, HOSINO 1942). Besides, even differences in details in size, shape and interrelation of individual spots are often under control of genic activity. These genes may belong to the same allelic series as the genes determining the major pattern types, or they may be entirely different ones (JOHNSON 1910, ZARAPKIN and TIMOFFÉEF-RESSOVSKY 1932, TENENBAUM 1933, HOSINO 1940–1943, SHULL 1944, 1945, 1948, 1949, TAN 1949). And for such variations, the presence of a directive tendency is often maintained (KELLOGG and BELL 1904, JOHNSON 1910, ZARAPKIN 1930, 1931, 1933, 1938, MODEREGGER 1933). The relative frequency of the genes controlling the elytral pattern, as well as the frequency of the genes governing their minute details, is geographically variable (JOHNSON 1910, DOBZHANSKY 1924, 1933, 1941, DOBZHANSKY and SIVERTZEW-DOBZHANSKY 1928, TIMOFFÉEF-RESSOVSKY 1932, KURISAKI 1927, ZIMMERMANN 1931, ZARAPKIN 1938, KOMAI, CHINO and HOSINO 1946, 1948, SHULL 1949). Furthermore, it has been demonstrated in some cases that such frequency shows seasonal fluctuations (TIMOFFÉEF-RESSOVSKY 1939, 1940, TAN 1949).

GENETICS OF ELYTRAL PATTERN IN *Harmonia*

HOSINO has been working on the genetics of this variable lady-beetle since 1933, and published his findings in his serial papers in the Japanese Journal of Genetics (1936–1948) and also in the Journal of Genetics (1940a). He held the view from the beginning that all differences among the major elytral pattern types are due to genes belonging to the same allelic series. TAN and

* Contribution from the NATIONAL INSTITUTE OF GENETICS, Japan.

LI (1934), who studied the same problem on Chinese material, once expressed the opinion that these patterns were governed by several different genes. In more recent papers, however, TAN (1946, 1949) admits the validity of the view of multiple allelism. HOSINO has further confirmed that even small variations within each major type, such as difference in size and shape, mode of appearance or disappearance, fusion or separation of individual spots, often have genetic bases. Thus, he has been able to distinguish the following numbers of subtypes within each major type:—*succinea*—10, *axyridis*—6, *aulica*—2, *conspicua*—4. All these differences are to be assigned to different alleles of one and the same locus. TAN also has arrived at practically identical results in his study on the Chinese brood of the same species, and he distinguishes the following subtypes:—*conspicua*—2, *spectabils*—2, *transversifacsia*—2. These two authors' classifications apparently have some coincidence. But, as far as can be judged from TAN's figures, the light areas in the elytral markings in most Chinese samples look considerably larger than in Japanese samples of the corresponding types. This difference probably has some relation to the difference in climatic conditions of the two countries.

GEOGRAPHIC VARIATION IN THE RELATIVE FREQUENCIES OF ELYTRAL PATTERN TYPES

DOBZHANSKY (1924, 1941) found very striking geographic variation in the relative frequency of the elytral pattern types of this lady-beetle collected at various localities in the Asiatic Continent and in Japan. His generalization on this subject is that "West-central Siberia (Altai, Yeniseisk) is occupied by a race manifesting nearly always the pattern *axyridis*. In central Siberia the yellow forms appear and rapidly displace *axyridis*, which on the Pacific Coast of Siberia and in China is very rare or is absent. *Spectabilis* and *conspicua* are found in the Far East only, the latter apparently reaching a high frequency in Japan. *Aulica* is nowhere frequent, but is found almost everywhere in the Far East" (DOBZHANSKY 1941, p. 69). Previous to DOBZHANSKY's study, CHINO and KOMAI compared their specimens of this species collected at two localities in Japan, Suwa in Nagano Prefecture and Tondabayasi near Osaka, and recognized a significant difference in the composition of the two samples (CHINO 1912). This material was supplemented by further collections of the beetles from the same localities, and also by those from several other localities (CHINO 1918). In 1927, KURISAKI carried out a similar study on the materials collected at Sapporo in Hokkaido, Tokyo, Gihu(Gifu) and Okayama in Honsyu, Matuyama in Sikoku, and at Hukuoka (Fukuoka) and Miyazaki in Kyusyu, and also Suigen in Korea. He found considerable variations among these materials. He noticed a general tendency in these variations that the light colored type, *succinea*, is relatively more abundant in the northern localities (Hokkaido and Korea), whereas the dark-colored types, *conspicua* and *spectabilis*, are relatively commoner in the southern localities (Sikoku and Kyusyu). This tendency, he remarks, is in reverse to the tendency observed in pupal coloration which becomes darker under low temperature.

TABLE 1

Percentages of different pattern individuals, and also percentage of the individuals provided with elytral ridge, among the materials from various localities. When more than two data are available from the same locality, the most recent datum is shown.

LOCALITY	YEAR	*succinea*	*axyridis*	*spectabilis*	*conspicua*	ELYTRAL RIDGE	TOTAL
Sapporo	'43, '44	42.9	1.0	21.6	34.3	99.5	1,184
Simamatu	'44	43.1	0.0	26.4	30.6	100.0	72
Akita	'44	60.0	2.2	9.6	28.2	42.4	135
Yamagata	'44, '45	34.0	5.9	10.0	50.2	53.8	253
Nikko	'14	47.3	3.5	8.2	41.0	—	451
Tutiura	'14	52.4	3.9	8.7	35.1	—	231
Takasino	'45	36.9	3.5	11.8	47.7	58.6	5,758
Tokyo	'42	34.3	3.9	7.3	54.5	55.6	178
Suwa	'42, '43	32.0	5.0	13.0	49.8	53.2	823
Matumoto	'13, '14	41.2	4.8	12.3	41.6	—	693
Nakatugawa	'16	30.3	5.9	16.4	47.3	—	152
Gihu	'40	19.0	4.8	11.7	64.4	41.6	272
Sanagé	'48, '49	23.1	14.5	25.9	36.2	34.7	24,443
Nagoya	'40	26.0	6.7	9.6	57.8	36.3	135
Terazu	'46	29.8	3.1	15.3	51.9	—	131
Kyoto	'40–'43	15.3	5.1	15.8	63.7	24.4	2,494
Osaka	'39	16.7	3.3	13.8	66.1	24.9	181
Tondabayasi	'43	16.0	5.7	10.3	68.0	23.8	194
Amagasaki	'40	13.7	3.4	10.9	71.9	20.5	386
Akasi	'44	13.3	5.4	15.0	66.3	18.8	240
Okayama*	'25	10.5	—	—	—	24.0	200
Kurasiki	'47	12.7	11.4	19.0	56.7	—	79
Onomiti	'44	12.3	4.1	13.7	69.9	18.8	219
Hirosima	'44	4.3	2.2	26.1	67.4	10.9	46
Matuyama	'44	10.7	5.8	19.1	64.1	11.2	534
Koti	'44, '45	9.4	8.6	20.1	61.9	8.0	673
Hukuoka	'44	2.3	2.2	11.1	83.6	12.1	995
Miyazaki*	'25	—	—	—	—	17.4	350
Suigen*	'25	85.8	—	—	—	97.0	402
Mukden	'44	90.7	0.0	4.5	4.6	96.5	1,865
Chihfeng	'44	82.4	0.0	12.1	4.4	98.9	91
Peiping†	?	83.3	0.0	8.9	7.3	—	9,635

In cases when the total percentage is smaller than 100, the deficient fraction is occupied by *aulica* and other rare types.

* KURISAKI's data.

† TAN & LI's data.

Recently, we obtained by the kindness of our friend biologists rather extensive materials of this beetle from various localities in Japan as well as in Manchuria. These specimens were collected mainly in the active season of this beetle from late spring to early summer. Only the materials from Suwa, Kyoto, Takasino in Japan, and those from Chihfeng and Mukden in Manchuria are hibernating groups. TAN (1949) states that he has observed in a population of this species near Hanchow a rather considerable seasonal

change in its composition. We have not noticed any such change so far in Japanese broods. If it is ascertained that such striking seasonal change as that found in the Chinese brood actually occurs in Japanese broods, our findings may require re-examination by taking this fact in consideration. Our findings on these materials are summarized in table 1, which includes KURISAKI'S, and TAN and LI'S data as well.

In this table it is shown that throughout Japan *succinea* (in which all forms with light-colored elytra are included) never exceeds 50 percent, and also

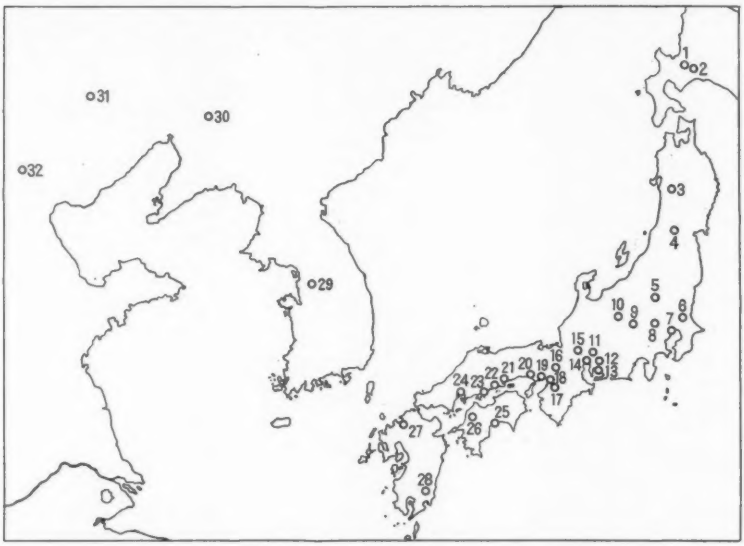

FIGURE 1.—The localities where the samples were collected: 1. Sapporo, 2. Simamatu, 3. Akita, 4. Yamagata, 5. Nikko, 6. Tutiura, 7. Tokyo, 8. Takasino, 9. Suwa, 10. Matumoto, 11. Nakatugawa, 12. Sanagé, 13. Terazu, 14. Nagoya, 15. Gihu, 16. Kyoto, 17. Tondabayasi, 18. Osaka, 19. Amagasaki, 20. Akasi, 21. Okayama, 22. Kurasiki, 23. Onomiti, 24. Hirosima, 25. Koti, 26. Matuyama, 27. Hukuoka, 28. Miyazaki, 29. Suigen, 30. Mukden, 31. Chihfeng, 32. Feiping.

that there is a gradual decrease in the relative frequency of *succinea* along the Japanese island chain from north-east to south-west. Thus, in Hokkaido the percentage of *succinea* exceeds 40, it decreases to 30–40 percent in Kanto District including Tokyo, and in Nagoya-Gihu District it comes down to 20–30 percent; in Kyoto-Osaka District the percentage becomes 15±, and in Tyugoku District (Okayama-Kurasiki-Onomiti-Hirosima) it is 10 percent; about the same frequency is found in Sikoku, whereas in Hukuoka in Kyusyu the frequency is as low as 2.3 percent. But, when one leaves the Japanese islands and goes across the strait to the Korean Peninsula, the percentage of *succinea* suddenly rises to 80+ and this high frequency is maintained through the localities in Manchuria to North China (fig. 1).

The corresponding gradient, though less marked, may be recognized in the relative frequencies of *spectabilis* and *conspicua*. Thus, *spectabilis* occupies

more than 20 percent of the Hokkaido populations; it is about 10 percent of Tohoku (Akita-Yamagata) and Kanto populations; it is 10–15 percent of Aiti (Nagoya and its vicinity) and Kyoto-Osaka populations, while in Tyugoku and Sikoku Districts it is about 20 percent, and the frequency decreases again to about 10 percent in the continent. Next, as for *conspicua*, 30 percent of the specimens of this species from Hokkaido have this pattern; this percentage is exceeded by the specimens from Nagoya-Gihu District; the percentage still rises in Kyoto-Osaka District to nearly 70. This frequency is maintained throughout Tyugoku and Sikoku, and the frequency attains its highest value, 80 percent in Kyusyu. On the continent *conspicua* is rare, its frequency is lower than 10 percent of the whole population. Designated by the incidence of the recessive gene for *succinea*, this gene is present in 65 percent of the gametes in the Hokkaido population; the value decreases in Kanto to 55–60 percent; it is 45–55 percent in Nagoya-Gihu, 40 percent in Kyoto-Osaka, 30 percent in Tyugoku and Sikoku, and in Kyusyu it is lower than 20 percent, whereas on the continent nearly 95 percent of all the elytral pattern genes are of *succinea*.

As for the type *axyridis*, such geographic variation is not found, its frequency remains uniformly at 5± percent throughout Japan. It may be noticed that this type is almost entirely missing from the Hokkaido populations, as well as from the continental populations. Another peculiar population is that of Sanagé, a village located about 30 kilometers east of Nagoya, which includes unusually many *axyridis* individuals. This population will be more crucially examined in another paper.

GEOGRAPHIC VARIATION IN THE FREQUENCY OF ELYTRAL RIDGE

Some individuals of this species have near the end of each elytron a small transverse ridge which can be recognized with a hand lens. This ridge is not found in other individuals, and its presence or absence is a clear-cut distinction. This fact was first noticed by KURISAKI (1927), who also has found that the frequency of the individuals having this ridge is geographically variable like the relative frequency of elytral patterns. Later, HOSINO (1936) confirmed that the presence or absence of this ridge is inherited on a monogenic basis, the gene for its presence being dominant over the gene for its absence. This gene is inherited independently of the gene controlling the elytral pattern. We have examined our materials in reference to this structural character also, and obtained the results presented in table 1 under the heading of "elytral ridge." One can see in this table that the beetles from Hokkaido are provided with this ridge almost without exception; the ridge is present in more than half of the individuals coming from the northern half of Honsyu, and in about 40 percent of those from Nagoya-Gihu District. The frequency comes down to 20–25 percent near Kyoto and Osaka, and goes on decreasing westward to become 10± in Sikoku and Kyusyu; but it suddenly rises to almost 100 percent in Korea, Manchuria and Jehol. Thus, we find in the frequency of the gene controlling this character, a geographic gradient parallel with the gradient in the relative frequency of elytral pattern genes. Designated by the frequency of the recessive gene for the absence of this ridge, less than

20 percent of the gametes of the individuals from Hokkaido carry this gene; the frequency increases to 70 percent in the northern districts of Honsyu, and goes on increasing westward to become 80 percent in Nagoya-Gihu District, nearly 90 percent in Kyoto-Osaka District and 95 percent in Sikoku and Kyusyu Districts.

TEMPORAL VARIATION IN THE RELATIVE FREQUENCIES OF ELYTRAL PATTERN TYPES

We have also some data on the relative frequencies of the elytral pattern types in samples collected at the same locality on different occasions between which there is sometimes a 15–30 year interval. These data are presented in table 2. Of these, the samples from Tondabayasi were collected by KOMAI, first in June 1911 and June 1913 and later in 1943 from wheat fields near the town. These samples indicate that there has been scarcely any change in this 30-year period in the composition of the population of this beetle at this locality. Next, the populations occurring near Tokyo were examined five times in the 28 years beginning from 1914. They were from different places in the northern suburbs of the city, a few kilometers apart from one another. These samples do not show any significant difference from one another, except the sample of 1917. This sample and also the one of 1914 were collected by KOMAI from wheat fields at Zyuzyo and at Sugamo respectively, which were then in the suburb of the city of Tokyo, and only 3 kilometers apart from each other. The sample of Sugamo is in composition much like the samples from other places near Tokyo. The sample of Zyuzyo, on the other hand, is somewhat peculiar in having more *spectabilis* and less *conspicua* than that of Sugamo, and the difference is statistically significant, thus:—

	SUGAMO SAMPLE	ZYUZYO SAMPLE	DIFFERENCE
Spectabilis	9.6±1.7%	23.1±3.9%	13.5±4.25%
Conspicua	52.1±2.9%	30.8±4.3%	21.3±5.14%

This, perhaps, is to be taken as a case of microgeographic variation rather than of temporal variation.

Next, both the samples of Matuyama and the samples of Hukuoka have a 19 year interval between 1925 and 1944 when they were collected. The composition of the earlier 1925 samples is not given besides the percentages of *succinea*. However, as far as it can be judged from these percentages as compared with the corresponding percentages in the samples of 1944, the populations of these two localities do not seem to have changed significantly in this period. The same is apparently true of the population of Gihu, because the difference between the samples of 1925 and 1940 from there is insufficient for being statistically significant.

In contrast to these samples, the samples of Sapporo collected in 1923 and in 1943–1944 respectively, show a sharp distinction in the relative frequency of *succinea*. While this type occupied 83.9 percent of the 1923 sample, it makes only 42.9 percent of the 1943–1944 sample. The former sample was examined by KURISAKI who gives the figure for *succinea* only. However, since he is a specialist on the taxonomy of Coccinellidae, there is no room for doubting

TABLE 2

*Temporal variation in the relative frequency of different pattern types and in the frequency of elytral ridge.**

LOCALITY	YEAR	*succinea*	*axyridis*	*spectabilis*	*conspicua*	ELYTRAL RIDGE	TOTAL
Sapporo	'23*	83.9	—	—	—	99.5	398
	'44	42.9	1.0	21.6	34.3	99.5	1,184
Suwa	'12, '13	42.6	4.6	9.5	42.3	—	2,005
	'14	41.7	5.6	10.9	41.8	—	1,413
	'15, '17	43.4	4.8	10.7	41.1	—	2,059
	'20	42.4	4.4	10.6	42.4	—	4,512
	'30	37.5	3.9	10.2	48.4	—	13,157
	'42, '43	32.0	5.0	13.0	49.8	53.2	823
Tokyo	'14	36.0	2.3	9.6	52.1	—	303
	'17	45.3	0.9	23.1	30.8	—	117
	'24	45.2	—	—	—	58.0	200
	'30, '31	39.6	3.7	11.0	45.8	52.0	2,283
	'42	34.3	3.9	7.3	54.5	55.6	178
Gihu	'23*	26.4	—	—	—	49.0	239
	'40	19.0	4.8	11.7	64.4	41.6	272
Tondabayasi	'11, '12	11.9	6.2	10.6	71.3	—	672
	'43	16.0	5.7	10.3	68.0	23.8	194
Matuyama	'25*	17.1	—	—	—	4.0	158
	'44	10.7	5.8	19.1	64.1	11.2	534
Hukuoka	'25*	3.1	—	—	—	25.0	229
	'44	2.3	2.2	11.1	83.6	12.1	995

* KURISAKI's data.

the accuracy of his classification of the four types in this species. Thus, it seems safe to conclude that there has been in these 20 years a significant change in the composition of the population of this beetle inhabiting Sapporo. This change implies a corresponding change in the frequency of the recessive gene for *succinea*, which decreased from circa 90 percent to 65 percent.

It is to be noticed that this figure for *succinea* in the older Sapporo sample resembles the one in the samples of Korea and Manchuria of the more recent date. The similarity of the fauna of Hokkaido to the fauna of the Asiatic Continent is known for various groups of land animals, and the Strait of Tugaru which separates Hokkaido from Honsyu is universally recognized as a zoogeographically important demarcation under the name of "Blakinston's Line." The composition of the recent sample of Sapporo, on the other hand, resembles the composition of the samples of the northern districts of Honsyū. The small sample of Simamatu, a village located about 20 kilometers southeast of Sapporo collected in 1944, is in composition nearly identical to the Sapporo sample in 1943–1944. When, however, these recent Hokkaido samples are more crucially examined, a remnant of their continental character becomes apparent, which is shown by the scarcity of *axyridis* and by the relatively high percentage of *spectabilis*.

A more distinct temporal change in the composition of population may be found in the six samples of hibernating colonies collected at Suwa by CHINO

in years ranging from 1912 to 1943. As shown in table 2, the population of this beetle in this locality has undergone hardly any change from 1912 to 1920. In the succeeding 10 years, however, it has changed somewhat, namely, the relative frequency of *succinea* has decreased, and that of *conspicua* has increased to a degree statistically significant in either case. This tendency persisted in the next 12 years, from 1930 to 1942 or 1943. During this period *succinea* lost 5.55 ± 1.68 percent, while *conspicua* gained 1.47 ± 1.80 percent; and of these changes that of *succinea* is statistically significant, thus:—

TYPE	YEAR			DIFFERENCE
	1912–1920 (A)	1930 (B)	1942, 1943 (C)	
Succinea	$42.66 \pm 0.49\%$	$37.51 \pm 0.43\%$	$31.96 \pm 1.62\%$	$A-B=5.15 \pm 0.65\%$ $B-C=5.55 \pm 1.68\%$
Conspicua	$42.04 \pm 0.49\%$	$48.35 \pm 0.42\%$	$49.82 \pm 1.74\%$	$B-A=6.31 \pm 0.66\%$ $C-B=1.47 \pm 1.80\%$

This change in the population of Suwa may be understood to mean that this population which formerly resembled the populations of Kanto District, has come in these 20 years nearer the populations of the Nagoya-Gihu District. Designated by the frequency of the gene for *succinea*, this change corresponds with circa 10 percent decrease of the gene.

TEMPORAL VARIATION IN THE FREQUENCY OF ELYTRAL RIDGE

A temporal variation similar to that found in the elytral pattern may be seen in the frequency of the elytral ridge as well. Thus, of the specimens from Hukuoka, 25 percent possessed this ridge in 1925, but it is found in only 12 percent of the specimens of 1944, and the difference is statistically significant. Among the specimens from Matuyama, on the other hand, the individuals provided with this ridge have increased in the same period from 4.0 percent to 11.2 percent, and the difference is also statistically significant, thus:

LOCALITY	YEAR		DIFFERENCE
	1925	1944	
Hukuoka	$25.0 \pm 2.86\%$	$12.1 \pm 1.72\%$	$25.0-12.1=12.9 \pm 3.40\%$
Matuyama	$4.0 \pm 1.55\%$	$11.2 \pm 1.37\%$	$11.2-4.0=7.2 \pm 2.06\%$

Designated by the frequency of the recessive gene for the absence of the ridge, the change in the Hukuoka population corresponds to about 7 percent decrease, and the change in the Matuyama population corresponds to about 5 percent increase.

The population of Gihu likewise appears to have changed somewhat in 17 years, but the difference is statistically insignificant. Such a change can not be recognized in other populations presented in the table. It may be added that, for all the older samples, KURISAKI'S data are referred to, to be compared with our own data. But, the presence or absence of this ridge is such a clear-cut distinction that scarcely any subjective judgement could appreciably influence the results of the examinations by different workers. It may also be noticed for the Sapporo population, that, in spite of the significant change in the relative frequencies of pattern type genes, no change has occurred in the frequency of the elytral ridge gene.

DISCUSSION

The geographic gradient observed in the proportion of the elytral pattern types and in the frequency of the elytral ridge in this beetle undoubtedly belong to the category of HUXLEY's "cline." The gradient passes along the Japanese island chain from north-east to south-west, undergoing a gradual change. A similar cline is recognized by GOLDSCHMIDT (1934, 1938, 1940) concerning various characters in Lymantria. These characters, according to him, vary in accordance with the local climatic conditions, especially with the difference in temperature. The gradient found in the relative frequencies of the pattern types in this beetle, however, proceeds apparently with little relation to the local temperature condition. It is a common experience of the students in the biology of lady-beetles that the size of the elytral markings is dependent on temperature and humidity to a great extent. The melanic marking is reduced under high temperature and low humidity and enlarged under low temperature and high humidity. The cline observed in the pattern types of Harmonia inhabiting Japan indicates that, as a general tendency, light-colored forms are found in smaller proportion in warmer southern districts than in colder northern districts.

A similar gradient is also found in the beetles of this species occurring on the Asiatic Continent, as well as in Korea. Thus, in KURISAKI's sample at Suigen, Korea, *succinea* occupies its 86 percent which resembles the figure 81.3 percent given by DOBZHANSKY (1927, 1933) for his sample of "Korea." Such high frequency of *succinea* is observed throughout Manchuria, Jehol and North China, as shown in TAN and LI's sample and ours. The frequency decreases southwards to become 66.6 percent in Soochow, and 42.6 percent in Szechwan (DOBZHANSKY 1941). TAN (1949) gives 48.6 percent for an April population and 58.5 percent for a November population of Hanchow in 1947. Thus, on the Asiatic Continent also, the cline goes rather in reverse direction to the temperature gradient. When, however, the humidity is considered, we find that the cline largely accords with the climatic gradient. DOBZHANSKY (1933, 1941), who studied the geographic variations in the elytral markings of several species of lady-beetles, has arrived at the conclusion that there is a tendency in this variation common to all these species, and that the variation largely accords with GLOGER-ALLEN's rule, the humidity being more effective than temperature on this variation.

To see if any differential vitality is found in relation to pattern types, the following observation was performed in the winters of 1942 and 1943. Certain numbers of beetles with different pattern types were selected from among four hibernating colonies collected in Kyoto or in Suwa, and they were kept in an unheated room in Kyoto through winter. The room temperature in this season in Kyoto sometimes comes down a few degrees below 0°C. The proportions of the individuals with different pattern types which died during winter before the end of April were compared with one another (table 3). The total number of beetles used was rather small. The general tendency, however, is obvious: the death rate of *axyridis* was significantly higher than that of other types among which no such difference was recognized.

TABLE 3

Death rate of individuals with different elytral pattern types during winter (%).

SOURCE OF SAMPLE	*succinea*	*axyridis*	*spectabilis*	*conspicua*	TOTAL NUMBER TESTED
Kyoto	14.0	39.1	21.0	21.8	378
Kyoto	22.4	40.0	16.7	24.6	496
Suwa	17.9	33.3	12.3	19.4	228
Suwa	16.0	35.7	23.5	19.8	255
Average	17.5±2.3	38.0±5.7	19.1±2.9	22.2±1.5	1,357

Axyridis−*succinea* =38.0−17.5=20.5±6.1
Axyridis−*spectabilis*=38.0−19.1=18.9±6.3
Axyridis−*conspicua* =38.0−22.2=15.8±5.9.

This finding, though meager, seems to have ascertained that selective mortality existed in connection with the *axyridis* pattern only, which is just in the middle in the grade of extension of melanic pigment, and that neither for the types with greater extension of the pigment, *conspicua* and *spectabilis*, nor for the type with smaller extension, *succinea*, was there any differential mortality during winter. TAN (1949) records his experience similar to this on a hibernating colony in Hanchow. His observation, however, seems to have substantiated that there was a selective mortality during the active season. If this seasonal variation is proved to be a regular one, it is perhaps due to the selective influence by humidity which shows considerable seasonal change in South China. This observation of TAN'S recalls TIMOFFÉEF-RESSOVSKY'S statement (1939, 1940, 1940b) on *Adalia bipunctata*, another variable lady-beetle. In a colony of this beetle hibernating at a certain place in Buch near Berlin this author found a regular cyclic change in the ratio between the black and red forms. This finding is different from TAN'S in that the selective mortality occurred during both the active and hibernating periods. At any rate, we can safely state that no apparent adaptive significance such as found in various characters in Lymantria can be attached to the difference in the elytral pattern of this beetle. The same is undoubtedly true for the presence or absence of the elytral ridge as well. Thus, both the cline in the elytral pattern and the cline in the elytral ridge concern a non-adaptive or neutral character. MAYR (1947) gives several instances of such clines.

In spite of this difference in the clines in Lymantria on the one hand and the clines in Harmonia on the other, one feature is common to both, namely, that Tugaru Strait marks a sharp gap. Of Lymantria, the strongest race in sex character occurs in Tohoku District, whereas the weakest race inhabits Hokkaido. Of Harmonia, nearly all the specimens from Hokkaido have the elytral ridge, while only about half of the specimens from Tohoku possess it. For the elytral pattern, such a gap is not found between the samples of Tohoku and the samples of Hokkaido obtained in 1943–1945. But, if we take the figure of 1923 as representing the original state of the Sapporo population, a similar contrast may be found between the populations of the two districts.

Next, a few previous cases where the composition of the same population of animals was examined on different occasions with a rather long time interval, may be cited. GEROULD (1940) ascertained that the ratio of white and yellow forms of the females of the pierid butterfly, *Colias philodice*, collected at Hanover, N.H. remained unchanged from 1911 to 1940. GORDON (1947) found that the proportion of six genes controlling the tail pattern of the tropical fish, *Platypoechilus maculatus* caught in certain places in Mexico and Guatemala, had remained nearly constant for more than 70 years from 1867 to 1939. One gene, however, increased somewhat from 1902 to 1939, while two genes decreased appreciably in the same period. In the common garden-snails of Europe, *Cepaea nemoralis* and *hortensis*, DIVER (1929) has ascertained that the proportion of the various color types has not changed significantly since the Pleistocene Period.

These are instances where Hardy-Weinberg's law of the constancy of gene composition within a random mating group has prevailed for a number of years. Among the cases where the composition has changed, the following record seems to resemble the present finding. KELLOGG and BELL (1904) observed in a population of a chrysomelid, *Diabrotica soror* inhabiting the campus of Stanford University, that there was a steady increase of the proportion of the individuals with fused elytral spots at the expense of those with separate spots from 1895 to 1904.

The cause of the temporal variation in the relative frequencies of elytral patterns in Harmonia stated above, remains obscure. But, there seems to be no doubt that human agency is responsible after all. It may be assumed for the Sapporo population, that the communication between Hokkaido and Honsyu has recently increased to such an extent that Tugaru Strait has lost its former efficiency of barring the exchange of the genes between the beetles inhabiting these districts. But this conjecture does not accord with the fact that the frequency of the elytral ridge has remained unchanged during the same period. Next, for the Suwa population, perhaps the Kiso Mountain Range had been an effective barrier between the population inhabiting Suwa Basin and that in the Nagoya-Gihu District. This barrier has been lifted by the recent development of traffic across the mountain range, and the gene flux between the populations of the insects inhabiting these two districts has been accelerated, and this may have brought the composition of the Suwa population somewhat nearer the state in the Nagoya-Gihu population. All these, however, are mere conjectures. The real cause remains to be elucidated.

SUMMARY

1. A geographic gradient (cline) is found in the relative frequencies of elytral pattern types and also in the frequency of the elytral ridge in the lady-beetle Harmonia inhabiting the Japanese islands. Neither of these characters seems to have any adaptive significance, and the cline passes apparently without any relation to climatic, especially temperature, gradients.

2. The clines are interrupted by an abrupt change at the Tugaru Strait between Hokkaido and Honsyu, and at the strait between Korea and Kyusyu.

The population of Hokkaido is characterized by its resemblance to the populations in Korea and North China.

3. Sets of samples collected at the same locality on occasions with an interval of from 15 to 30 years between, were secured from several localities in Japan. Some of them show that there was practically no change in its composition during this period; but a few others indicate an obvious change in the composition of the population.

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